Antioxidant vitamin–cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials[1,2]

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ABSTRACT The hypothesis that antioxidant vitamins might decrease the risk of cardiovascular disease (CVD) is a promising area of research. At present, however, it is far from certain whether antioxidant vitamins confer protection against CVD. Evidence for the antioxidant vitamin–cardiovascular disease hypothesis has accumulated from several lines of research. Laboratory research has identified biochemical properties of antioxidant vitamins that could explain their possible role in inhibiting and delaying coronary atherosclerosis. Epidemiologic studies have provided support for the hypothesis by showing that people who consume high amounts of antioxidant vitamins through diet or supplements, or those with high concentrations of these nutrients in their blood, tend to have lower risks of CVD. In the case of the former, however, laboratory findings may not have relevance to free-living humans. Observational epidemiologic studies cannot exclude the possibility that people who consume antioxidant-rich diets or who take vitamin supplements also share other lifestyle or dietary practices that actually account for their lower disease rates. Because of these uncertainties, the only way to determine reliably whether antioxidants play any role in reducing the risk of CVD is to conduct large-scale, randomized trials of these agents, in which adequate doses of antioxidant vitamins are tested for a sufficient duration to allow for any benefits to emerge. Several large-scale trials are now ongoing in both primary and secondary prevention. The results of these trials over the next several years should provide reliable evidence for this promising, but as yet unproven, hypothesis. *Am J Clin Nutr* 1995;62(suppl):1377S–80S.

INTRODUCTION

As is the case with antioxidants and cardiovascular disease (CVD), advances in medical knowledge proceed on several fronts, optimally simultaneously (1). Basic researchers provide answers to the question of why an agent may reduce premature death. Clinicians provide enormous benefits to affected patients through applications of advances in diagnosis and treatment, and formulate many hypotheses from their clinical experiences in case reports and case series. Epidemiologists and biostatisticians formulate hypotheses from descriptive studies and test hypotheses in analytic studies to answer the crucial question of whether an agent actually reduces risk of disease. Research strategies to test hypotheses include observational analytic studies (case-control or cohort) and randomized clinical trials. Every discipline and, indeed, each strategy within a discipline, contributes important relevant and complementary information to a totality of evidence on which we can base more rational clinical decisions for individual patients and policy decisions for the health of the general public.

BASIC RESEARCH AND OBSERVATIONAL EPIDEMIOLOGY

With respect to basic research findings, several mechanisms have been proposed whereby antioxidant vitamins may decrease risks of CVD. Vitamin E inhibits oxidation of low-density-lipoprotein (LDL) cholesterol, a particularly atherogenic molecule (2, 3). In contrast, β-carotene may prevent endothelial damage by inhibiting oxidization of LDL within the tissue (4). Thus, different antioxidants may have different principal modes of action in decreasing risks of CVD.

In epidemiologic research, descriptive studies (5, 6) contributed to the formulation of the hypothesis that antioxidant vitamins may decrease risks of CVD. Some, but not all, observational analytic studies, carried out in Europe (7–10) and later in the United States (11–14), have tended to show that individuals who consume large amounts of antioxidant vitamins through diet or supplements have lower risks of CVD than those who do not.

THE NEED FOR LARGE-SCALE, RANDOMIZED TRIALS

For most hypotheses, randomized trials are neither necessary nor desirable (15). For example, the introduction of penicillin to treat pneumococcal bronchopneumonia or antihypertensive drug therapy for malignant hypertension led to benefits that were so large and obvious that randomization of patients was unnecessary and, indeed, would have been unethical. Increasingly, however, the most plausible benefits of many promising interventions are small to modest in size—with hypothesized

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risk reductions of perhaps 20–40%. In such cases, the amount of uncontrolled and uncontrollable confounding inherent in observational study designs is about as large as the effects being sought (16). For antioxidant vitamins, even modest systematic differences between those who consume large and small amounts of these micronutrients could well account for the small-to-moderate protection against CVD associated with these nutrients in observational studies. For these reasons, only randomized trials of adequate sample size, dose, and duration of treatment and follow-up can determine reliably whether there are in fact small to moderate—but clinically worthwhile—benefits of antioxidant vitamins. This perspective has been reinforced by the summary statement from the 1991 National Heart, Lung and Blood Institute consensus conference "Antioxidants in the Prevention of Human Atherosclerosis," which supported the need for randomized clinical trials of β-carotene, vitamin E, and/or vitamin C in primary and secondary prevention of CVD (17).

COMPLETED LARGE-SCALE, RANDOMIZED TRIALS

Only two large-scale, randomized trials have been completed testing antioxidant vitamins, both of which were designed to test these agents in cancer prevention. The Alpha-Tocopherol Beta-Carotene Cancer Prevention Study involved 6 y of randomized treatment with 20 mg β-carotene (Solatene; Hoffmann-La Roche Inc, Nutley, NJ) and/or 50 mg vitamin E (synthetic form) daily in 29 133 Finnish male smokers, aged 50–69 y (18). There was no apparent benefit of either vitamin on cardiovascular outcomes. In fact, those assigned to β-carotene experienced a small, but significant, 11% increase in ischemic heart disease mortality, whereas those assigned to vitamin E experienced a statistically significant 50% higher rate of cerebral hemorrhages. With respect to cancer endpoints, no protective effect on lung cancer was observed for either of the two vitamins. In fact, those assigned to β-carotene had a statistically significant 18% higher risk of lung cancer, a finding greatly at variance with the totality of other evidence suggesting a possible benefit. For vitamin E, there were lower risks of prostate and colon cancer, but these hypotheses had not been specified a priori.

The other completed large-scale, randomized trial of antioxidants is the Chinese Cancer Prevention Trial, in which 29 584 residents of four communities in Linxian, a rural county in north-central China were randomly assigned to receive one of eight different vitamin-mineral supplement combinations (19). The Linxian region suffers from one of the world's highest rates of esophageal cancer, and dietary intake of most micronutrients is very low. Nine different agents were tested in this study: retinol, zinc, riboflavin, niacin, vitamin C, molybdenum, β-carotene, vitamin E, and selenium. For subjects receiving the combined daily treatment of β-carotene (15 mg), vitamin E (30 mg), and selenium (50 μg), there was a nonsignificant 10% decrease in cerebrovascular mortality. Because most strokes in this population are likely to be hemorrhagic rather than thromboembolic, the relevance of this finding to the prevention of atherosclerosis is unclear. It was not possible to assess any effects on coronary heart disease (CHD), because this disease is so uncommon in this population. For other outcomes, total mortality was 9% lower among those receiving this vitamin-mineral combination than among those not receiving this treatment, with a significant 21% decrease in gastric cancer deaths. Because nutrients were studied in combined groups, it is impossible to distinguish the relative contributions of β-carotene, vitamin E, and selenium to any observed finding. Moreover, the relevance of findings on vitamin supplementation in a poorly nourished population to a population with adequate intake of vitamins and minerals is unclear.

The only other randomized trial evidence available to date on vitamin E includes several small trials of only 6 mo duration, the results of which, not surprisingly, are inconsistent (20). With respect to β-carotene, a subgroup analysis has been conducted among 333 men in the US Physicians' Health Study who reported at baseline a history of chronic stable angina, a prior coronary revascularization procedure, or both (21). After 5 y of treatment, those assigned to β-carotene (50 mg every other day, supplied as Lurotin by BASF AG, Ludwigshafen, Germany) experienced a significant 54% reduction in subsequent vascular events (nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or cardiovascular death). This apparent benefit was not evident until the second year of treatment, a finding compatible with an effect of β-carotene on atherosclerosis. Further, of 27 incident myocardial infarctions, there were 7 in the aspirin group and 20 in the placebo group, and 10 in the β-carotene group and 17 in the β-carotene placebo group. Among subjects assigned at random to both aspirin and β-carotene, there were no myocardial infarctions, a finding compatible with the acute benefits of aspirin on thrombosis and the more chronic effects of β-carotene on atherosclerosis. The number of endpoints in this subgroup analysis was small and the 95% CIs wide, but this intriguing finding merits further testing in randomized trials of primary and secondary prevention.

ONGOING LARGE-SCALE, RANDOMIZED TRIALS

Reliable evidence on the balance of benefits and risks of antioxidant vitamins in CVD will emerge from several large-scale, randomized trials that are now ongoing in well-nourished populations. Three of these trials are being conducted by our research group at Brigham and Women's Hospital in Boston. The Physicians' Health Study, which began in 1982, is testing β-carotene (50 mg on alternate days in the form of Lurotin, supplied by BASF AG) in 22 071 US male physicians (22). The Women's Health Study, which began in 1992, plans to randomly assign ≈40 000 apparently healthy US women by using a 2 × 2 × 2 factorial design to evaluate the benefits and risks on cardiovascular disease and cancer of β-carotene (50 mg on alternate days, in the form of Lurotin, supplied by BASF AG), vitamin E [600 IU (436 mg) on alternate days, supplied by the Natural Source Vitamin E Association, La Grange, IL], and low-dose aspirin (100 mg on alternate days, supplied by Bayer Corporation, Parsippany, NJ) (23). Finally, in secondary prevention, the Women's Antioxidant and Cardiovascular Study will randomly assign ≈8000 women not eligible for the Women's Health Study because of a prior history of cardiovascular disease to test the benefits and risks of β-carotene (50 mg on alternate days, in the form of Lurotin, supplied by BASF AG), vitamin E [600 IU (436 mg) on alternate days, supplied by the Natural Source Vitamin E Association], and vitamin C

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TABLE 1
Ongoing trials of antioxidant vitamins and cardiovascular disease

Study and location	Agents	Population
United States		
Physicians' Health Study	β-Carotene	US male physicians at usual risk
Women's Health Study	β-Carotene, vitamin E, and low-dose aspirin	US female health professionals at usual risk
Women's Antioxidant and Cardiovascular Study	β-Carotene, vitamin E, and vitamin C	US female health professionals with prior history of cardiovascular disease
Carotene and Retinol Efficacy Trial	β-Carotene and retinol	US men with occupational asbestos exposure, history of cigarette smoking, or both
Canada		
Heart Outcomes Prevention Evaluation Study	Vitamin E and ACE inhibitors[1]	High-risk patients
United Kingdom		
Heart Protection Study	β-Carotene, vitamin E, vitamin C, and simvastatin	Patients with prior coronary disease, occlusive disease of noncoronary arteries, or diabetes mellitus
Europe		
Supplementation en Vitamines et Mineraux Antioxydants Trial	β-Carotene, α-tocopherol, vitamin C, selenium, and zinc	Men and women at usual risk

[1] Angiotensin converting enzyme.

(500 mg daily, provided by BASF AG) (24). In designing each of these trials, considerable attention was given to the need for an adequate sample size, dose, and duration of treatment and follow-up to allow for the emergence of any effect of these antioxidant vitamins.

Size

Although small-to-moderate benefits of antioxidant vitamins on CVD would be clinically worthwhile and have a large public health effect, they are difficult to detect reliably. For statistically significant results to emerge, small-to-moderate systematic effects must be greater than random errors and, hence, the need for large sample sizes. Further, although randomization achieves a degree of control of confounding not possible in observational studies, this can only be achieved with large sample sizes. In secondary prevention, this requires randomization of at least thousands of patients and, in primary prevention, tens of thousands of persons. For these reasons, the Women's Antioxidant and Cardiovascular Study secondary prevention trial plans to enroll ≈8000 women with prior cardiovascular disease events. In primary prevention, 22 071 male physicians have been randomly assigned in the Physicians' Health Study. In the Women's Health Study, because of the lower age-specific CVD rates in women, plans are to randomly assign ≈40 000 female health professionals.

Dose

In observational studies, subjects are often divided into quintiles based on antioxidant vitamin intake. Comparisons are usually then made between the highest and lowest quintiles. In contrast, in randomized trials the antioxidant vitamin intake in placebo group subjects will reflect the distribution of intakes in the general population. To achieve sufficient differences in intake between the treatment and comparison groups, therefore, the active treatment groups need to be given doses high enough to put subjects into the top few percentiles of intake, not merely the top quintile. At the same time, trials must use doses of antioxidant vitamins that will not cause any real or perceived adverse effects that might lower compliance.

In the three antioxidant trials we are conducting, subjects will receive 50 mg β-carotene on alternate days (as Lurotin, supplied by BASF AG). This dose is roughly bioequivalent to 15 mg Solatene/d (manufactured by Hoffmann-La Roche) and is sufficient to place individuals in the top few percentiles of β-carotene intake without producing skin discoloration (25). In contrast, the daily dose of 20 mg Solatene used in the Alpha-Tocopherol Beta-Carotene trial produced skin discoloration that may have contributed to decreased compliance. For vitamin E, in the Women's Health Study and Women's Antioxidant and Cardiovascular Study we chose a dose of 600 IU (436 mg) α-tocopherol acetate on alternate days (supplied by the Natural Source Vitamin E Association). In the Alpha-Tocopherol Beta-Carotene trial a 50-mg daily dose of vitamin E in a synthetic form was used, which may be less bioavailable than the natural source and was far lower than the ≥ 100-mg daily doses that were associated with CVD benefits in observational studies. For vitamin C, we chose to test in the Women's Antioxidant and Cardiovascular Study a daily 500-mg dose (supplied by BASF AG) to achieve concentrations suggested to have possible benefits on CVD.

Duration of treatment and follow-up

Regarding the necessary duration of treatment and follow-up in trials of antioxidants, the analogy in observational studies with smoking cessation on risks of lung cancer and CHD suggests that there may be a lag time of several years for CHD and of perhaps 8–10 y for cancer before full benefits are evident. The Finnish Alpha-Tocopherol Beta-Carotene trial, with 6 y of treatment and follow-up, would appear to have had a sufficient length of treatment to detect an effect on cardiovascular outcomes. However, this length of time may have been inadequate to yield a clearly detectable reduction in cancer, which is a multistage process that often proceeds over a decade or more (26). In contrast, the Physicians' Health

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Study, which will end in late 1995, will have an average duration of treatment and follow-up of ≈13 y and should, therefore, provide reliable data on β-carotene in the prevention of cancer as well as CVD.

In addition to the three trials we are conducting, several other large-scale, randomized trials of antioxidants are ongoing. In the Carotene and Retinol Efficacy Trial a combined daily regimen of 25 mg β-carotene and 25 000 IU retinoic acid is being tested in 18 000 US men at high risk for lung cancer because of occupational asbestos exposure, cigarette smoking, or both. In Canada, the Heart Outcomes Prevention Evaluation Study is testing vitamin E and drugs that inhibit angiotensin converting enzyme in 9000 patients at high risk for heart disease. In the United Kingdom, the Heart Protection Study is testing daily doses of vitamin C (250 mg), vitamin E (600 mg), β-carotene (20 mg), and simvastatin, a 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor, in 20 000 patients at high risk for heart disease because of prior evidence of coronary disease, occlusive disease of noncoronary arteries, or diabetes mellitus. Finally, a French study, the Supplementation en Vitamines et Mineraux Antioxydants trial, is testing a combined daily regimen of relatively low doses of vitamins and minerals (6 mg β-carotene, 15 mg α-tocopherol, 120 mg vitamin C, 100 μg selenium, and 20 mg zinc) in 15 000 healthy men and women. Currently ongoing large-scale, randomized trials of antioxidant vitamins are listed in **Table 1**.

CONCLUSION

The results of these large-scale, randomized trials will contribute important relevant and complementary information to ongoing basic research, clinical investigation, and observational studies. Over the next several years, this information should yield a totality of evidence sufficient for making rational clinical decisions for individual patients and policy decisions for the health of the general public concerning antioxidant vitamins and cardiovascular disease.

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