Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials1,2

Joachim Bleys, Edgar R Miller III, Roberto Pastor-Barriuso, Lawrence J Appel, and Eliseo Guallar

ABSTRACT

Background: Laboratory and observational studies suggest that antioxidant and B vitamin supplementation may prevent atherosclerosis. Although trials have not shown a benefit of these supplements on clinical cardiovascular events, it is unknown whether they affect the progression of atherosclerosis as measured by imaging techniques.

Objective: The objective was to perform a meta-analysis of randomized controlled trials of the effect of vitamin-mineral supplementation on atherosclerosis progression.

Design: We searched the MEDLINE, EMBASE, and CENTRAL databases for relevant studies. No language restrictions were applied. We separately analyzed trials using antioxidants (vitamins E and C, β-carotene, or selenium) and trials using B vitamins (folic acid, vitamin B-6, or vitamin B-12). The progression of atherosclerosis was evaluated by B-mode ultrasound, intravascular ultrasound, or angiography. Effect sizes were calculated for the difference in slope of atherosclerosis progression between participants assigned to supplements and those assigned to the control group.

Results: In trials not involving percutaneous transluminal coronary angioplasty, the pooled effect size was −0.06 (95% CI: −0.20, 0.09; 7 trials) for antioxidants and −0.93 (95% CI: −2.11, 0.16; 4 trials) for B vitamins. In trials involving percutaneous transluminal coronary angioplasty, the pooled relative risk of restenosis was 0.82 (95% CI: 0.54, 1.26; 3 trials) for antioxidants and 0.84 (95% CI: 0.34, 2.07; 2 trials) for B vitamins.

Conclusion: Our meta-analysis showed no evidence of a protective effect of antioxidant or B vitamin supplements on the progression of atherosclerosis, thus providing a mechanistic explanation for their lack of effect on clinical cardiovascular events. Am J Clin Nutr 2006;84:880–7.

KEY WORDS Meta-analysis, antioxidants, folate, B vitamins, atherosclerosis, prevention

INTRODUCTION

Vitamin and mineral supplements have been promoted as a strategy to prevent atherosclerosis. In vitro studies have shown that antioxidants such as vitamins E and C, β-carotene, and selenium reduce lipid peroxidation and free radical damage, which are important intermediaries in the pathogenesis of atherosclerosis (1–4). B vitamins, including folate, vitamin B-6, and vitamin B-12, lower homocysteine concentrations (5, 6)—an independent cardiovascular disease risk factor in observational studies (7, 8). In addition, a low dietary intake of antioxidant vitamins was associated with greater rates of progression of atherosclerosis in observational studies (9).

On the basis of this laboratory and observational evidence, randomized controlled trials were conducted to assess the effects of vitamin-mineral supplements. The supplements tested so far have failed to show a protective effect on clinical cardiovascular endpoints or mortality (10, 11). The direct effect of vitamin-mineral supplementation on the progression of atherosclerosis, however, is unknown. It is possible that supplements might retard the progression of atherosclerosis but have no effect on cardiovascular clinical events because of a concomitant deleterious effect, such as a hemorrhagic effect (12).

The objective of this meta-analysis was to synthesize the evidence from randomized controlled trials performed specifically to assess the effect of vitamin-mineral supplementation on the progression of atherosclerosis measured through imaging techniques, including angiography, ultrasonography, magnetic resonance imaging, and computed tomography.

METHODS

Literature search and study selection

The search strategy for this study was part of a larger project to review the effects of any dietary supplement on the progression of subclinical atherosclerosis in humans. The complete list of supplements was obtained from the Office of Dietary Supplements of the US National Institutes of Health (13). We searched MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL) for randomized controlled trials of dietary supplements and atherosclerosis using the search strategy detailed in the online supplement (see Appendix under “Supplemental data” in the current issue at www.ajcn.org). The search

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was updated through March 2006. There were no restrictions on language or year of publication.

From the search, we sought to identify all randomized clinical trials that assessed vitamin-mineral supplements and progression of subclinical atherosclerosis. We were as inclusive as possible in our selection criteria, because we tried to identify randomized controlled trials of any type of vitamin-mineral supplement (irrespective of compositional elements, dose, or form of administration) that evaluated the progression of subclinical atherosclerosis in any vascular territory and assessed through any imaging technique. The a priori defined exclusion criteria were as follows: 1) no data in humans, 2) no original data, 3) observational epidemiologic study, 4) no random allocation, 5) no vitamin-mineral supplement as intervention, and 6) no subclinical marker of atherosclerosis as outcome. In addition, we excluded one trial that compared a combination of a vitamin supplement plus atorvastatin to placebo (14) and 3 trials that did not report rates of progression of atherosclerosis (15–17). When the same study originated several reports, we used the findings of the report included all participants or the report with the longest follow-up. The study selection process is summarized in Figure 1.

All randomized trials identified through this process tested either antioxidants (vitamins E and C, β-carotene, or selenium) or B vitamins (folate, vitamin B-6 or vitamin B-12). Because antioxidants and B vitamins are presumed to prevent the progression of atherosclerosis through different mechanisms, we analyzed these 2 groups of trials separately.

Several of the trials identified evaluated the efficacy of supplements on lesion progression after percutaneous transluminal coronary angioplasty (PTCA). Because the pathophysiologic mechanisms involved in lesion progression may be different in post-PTCA patients than in subjects who did not undergo PTCA, we also separated post-PTCA studies from no-PTCA studies.

### Data abstraction and study outcomes

Two investigators (JB and EG) independently read the titles and abstracts identified in the search and retrieved articles to determine eligibility and to extract study data. Discrepancies were resolved by consensus.

For studies in subjects not undergoing PTCA, the outcome was the difference in slope of atherosclerosis progression between participants assigned to vitamin-mineral supplementation and those assigned to the control group. Progression of atherosclerosis was defined as the progression of intima-media thickness assessed by ultrasound, the reduction in minimal luminal diameter (MLD) assessed by angiography, or the progression in maximum intimal thickness or intimal area assessed by intravascular ultrasound.

For post-PTCA studies, we assessed the percentage of patients with restenosis at the end of follow-up (restenosis rate). In addition, we also assessed the progression of atherosclerosis, defined as the change in lumen diameter of dilated artery segments determined angiographically (late loss).

### Statistical analysis

Because the methods for evaluating the progression of atherosclerosis varied across studies, we used effect sizes to obtain standardized measures of effect for the difference in the progression of atherosclerosis by comparing supplement with control groups. Effect sizes were computed by dividing the difference in slope for progression by their pooled SD (Cohen’s d) (18). Effect sizes are interpreted as the effect of vitamin-mineral supplementation on the progression of atherosclerosis measured in SDs. For the post-PTCA studies, we also computed the relative risk of restenosis in those assigned to vitamin-mineral supplements compared with control subjects.

Pooled estimates and 95% CIs of effect sizes and relative risks were calculated by using an inverse-variance weighted random-effects model. Between-study heterogeneity was quantified by using the I² statistic (19). Statistical analyses were performed by using STATA 8.2 (Stata Corp, College Station, TX).

### RESULTS

#### No-PTCA studies

Eleven randomized controlled trials of vitamin-mineral supplements and atherosclerosis progression in subjects who had not undergone PTCA met our inclusion criteria (Table 1) (20–30). One trial targeted subjects at low risk of atherosclerotic disease (26), 3 trials assessed subjects at high risk of atherosclerotic disease (23, 25, 30), 4 trials evaluated patients with evidence of atherosclerotic disease (20, 21, 24, 28), 1 trial was conducted in renal transplant patients (27), and 2 trials were conducted in cardiac transplant patients (22, 29). The average follow-up varied between 0.5 and 7.2 y. All trials except for one (29) were double-blind.

Seven trials used antioxidants (20–26); the total number of patients in these trials was 3130. The sample sizes of individual trials ranged from 37 to 1162. All antioxidant trials included vitamin E as part of the treatment regimen. Two trials used vitamin E alone (21, 23); 3 trials used vitamin E in combination with vitamin C (22, 24, 25); 1 trial used a combination of vitamins E and C, selenium, and β-carotene (20); and 1 trial used a combination of vitamins E and C, selenium, β-carotene, and zinc (26). The pooled effect size for the progression of atherosclerosis in a comparison of antioxidants with control treatments was −0.06 (95% CI: −0.20, 0.09; P = 0.44; Figure 2). After the trial
### TABLE 1
Trials of vitamin-mineral supplements for the prevention of the progression of atherosclerosis in subjects who did not undergo percutaneous transluminal coronary angioplasty

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<th>Follow-up</th>
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<th>Double-blind</th>
<th>Factorial design</th>
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1 angio, angiography; CVD, cardiovascular disease; HRT, hormone replacement therapy; IMT, intima-media thickness; IU, International units; IVUS, intravascular ultrasound; MLD, minimal lumen diameter; NA, not available; US, ultrasound.

2 The factorial interventions for trials with a factorial design are shown in parentheses.

3 The control group in the VISP trial received a very low dose of B vitamins (0.02 mg folate, 0.2 mg vitamin B-6, and 6 µg vitamin B-12).
performed in cardiac transplant patients was excluded, the pooled effect size was −0.02 (95% CI: −0.15, 0.10; \( P = 0.72 \)).

There was significant heterogeneity among the trials, even after exclusion of the trial in cardiac transplant patients (\( I^2 = 62\% \); 95% CI: 9%, 84%). However, the small number of trials limited a detailed evaluation of the sources of heterogeneity.

Four trials tested B vitamins (27–30). All of these trials used folate, either alone (29) or in combination with vitamin B-6 and B12 (27, 28, 30). The total number of participants in these trials was 260. The sample sizes of individual trials ranged from 44 to 110. The pooled effect size for atherosclerosis progression in a comparison of B vitamin supplementation with control treatment was −0.93 (95% CI: −2.11, 0.26; \( P = 0.12 \); Figure 3).

After exclusion of one trial in cardiac transplant patients, the pooled effect size was −1.20 (95% CI: −2.87, 0.47; \( P = 0.16 \)) with substantial heterogeneity (\( I^2 = 96\% \); 95% CI: 92%, 98%).

Post-PTCA studies

Five randomized controlled trials of vitamin-mineral supplements on the progression of post-PTCA lesions met our inclusion criteria (Table 2) (31–35). Two trials allowed for stenting (34, 35), 2 trials evaluated only patients without stenting (32, 33), and 1 trial did not provide information on stenting (31). Average follow-up varied between 0.3 and 0.5 y. All trials except for one (32) were double-blind.

Three studies, with a total of 536 patients (range in individual trials: 85–230), assessed antioxidant supplements: 1 used vitamin C alone (32), 1 used vitamin E alone (31), and 1 used a combination of vitamins E and C and \( \beta \)-carotene (33). The pooled relative risk of restenosis in a comparison of antioxidants with control treatments in post-PTCA patients was 0.82 (95% CI: 0.54, 1.26; \( P = 0.37 \); Figure 4). Two studies, with a total of 660 patients, assessed a combination of folate, vitamin B-6, and vitamin B-12 (34, 35). For B vitamin supplementation, the pooled relative risk of restenosis was 0.84 (95% CI: 0.34, 2.07; \( P = 0.98 \)). The pooled effect sizes for late loss in these trials were consistent with the relative risks of restenosis (Figure 4).

DISCUSSION

Our meta-analysis showed no evidence of a protective effect of antioxidant vitamin-mineral or B vitamin supplementation on the progression of atherosclerosis. Also, neither antioxidant supplements nor B vitamins prevented late loss or restenosis after PTCA. Our findings add to recent skepticism about the presumed beneficial effects of vitamin-mineral supplementation on clinical cardiovascular endpoints.

Antioxidant supplements

Extensive biological research supports a key role of oxidative stress on atherogenesis. According to the oxidative-modification hypothesis, free radical damage induces oxidative changes to LDL particles that initiate and promote atherosclerotic changes (4, 36). Because vitamin E is fat soluble and is integrated in LDL particles, it was expected to be particularly protective (37). It was expected that other antioxidants would also protect against atherosclerosis (36).

Indeed, observational studies that evaluated the association between measures of intake or biomarkers of antioxidants have frequently shown inverse associations with clinical cardiovascular events (38). Randomized clinical trials, however, have clearly shown a lack of benefit of \( \beta \)-carotene and vitamin E on clinical cardiovascular events, with some trials actually showing a small but significant increase in cardiovascular events and all-cause mortality (11, 39).

Our meta-analysis showed no compelling evidence that antioxidant supplements prevent the progression of atherosclerosis. Of 6 no-PTCA trials using antioxidant vitamins in nontransplant patients, 4 relatively large trials found either neutral or possibly harmful effects on atherosclerosis progression (21, 24, 26, 27). 1 additional trial showed that antioxidants attenuated the protective effect of simvastatin-niacin on the progression of atherosclerosis when used in combination (20). All these trials were placebo controlled, double-blind, and randomized, which are considered characteristics of high-quality trials in standard quality scores (40). Although the methods used to evaluate the progression of atherosclerosis were heterogeneous, similar methods did detect a benefit on atherosclerosis progression in trials of statins (41). It is thus unlikely that a substantial benefit of antioxidants would have been missed in our analysis.
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<th>Control arm</th>
<th>n</th>
<th>Dosed</th>
<th>Drops</th>
<th>Assessed outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DeMaio et al, 1992 (31)</td>
<td>United States</td>
<td>NA 82</td>
<td>54</td>
<td>0.3</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Vitamin E</td>
<td>45</td>
<td>1200 IU</td>
<td>40</td>
<td>26</td>
</tr>
<tr>
<td>Tomoda et al, 1996 (32)</td>
<td>Japan</td>
<td>No 81</td>
<td>60</td>
<td>0.3</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Vitamin C</td>
<td>50</td>
<td>500 mg</td>
<td>51</td>
<td>15</td>
</tr>
<tr>
<td>MVP, 1997 (33)</td>
<td>Canada</td>
<td>No 77</td>
<td>59</td>
<td>0.5</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes (propranol)</td>
<td>Vitamin E</td>
<td>110</td>
<td>1400 IU/1000 IU/60 000 IU</td>
<td>120</td>
<td>27</td>
</tr>
<tr>
<td>Folate and vitamins B-6 and B-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Swiss Heart Study, 2001 (34)</td>
<td>Switzerland</td>
<td>Yes (56%) 78</td>
<td>61</td>
<td>0.5</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Folate B-6</td>
<td>92</td>
<td>1 mg</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td>Lange et al, 2004 (35)</td>
<td>Germany, Netherlands</td>
<td>Yes (100%) 77</td>
<td>61</td>
<td>0.5</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Folate B-6</td>
<td>242</td>
<td>1.2 mg</td>
<td>241</td>
<td>24</td>
</tr>
</tbody>
</table>

1 IU, International units; NA, not available.
2 The outcomes in all trials were assessed by angiography. The cutoffs used to define restenosis in the original studies are shown in parentheses.
Although observational studies showed a positive association between homocysteine concentrations and cardiovascular events (7), randomized trials have so far failed to show a beneficial effect of B vitamin supplements on cardiovascular disease outcomes (10). The effect of B vitamins on the progression of atherosclerosis has only been addressed in a few small trials, and their results were highly heterogeneous. As a consequence, the random-effects CI for the pooled effect size was wide. Because absolute measurements of atherosclerosis progression were not available, we could not incorporate the findings of a 2-y placebo-controlled trial of folate (5 mg/d) plus vitamin B-6 (250 mg/d) in our quantitative meta-analysis. Instead, this trial reported the odds ratios for progression of femoral and carotid atherosclerosis by duplex ultrasound in 158 randomized participants (16). B vitamins had no apparent effect on these outcomes. The odds ratios for atherosclerosis progression in a comparison of the intervention with placebo were 1.02 (95% CI: 0.26, 4.05) for femoral atherosclerosis and 0.86 (95% CI: 0.47, 1.59) for carotid atherosclerosis. In a separate report from the same study, the odds ratio for the progression of carotid artery stenosis assessed by magnetic resonance angiography in a comparison of B vitamins with placebo was 0.48 (95% CI: 0.17, 1.41; n = 141) (17). In this trial, progression of atherosclerosis was available only categorically, and there was no information on progression rates to estimate effect sizes. More precise estimates of the effect of B vitamins on atherosclerosis progression will require large high-quality randomized trials.

### B vitamins

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### Post-PTCA studies

Restenosis is a major factor that limits the benefits of PTCA (42). Mechanistic and animal studies support a potential benefit of antioxidant and B vitamin supplements on restenosis after PTCA or stenting (43–46). In this meta-analysis we observed no benefit of antioxidant supplementation on restenosis after PTCA, but the evidence was based on only 3 small or moderately sized trials. The largest study of antioxidant vitamins in post-PTCA patients, however, showed no effect (33). For B vitamins, 2 moderately large randomized trials had significant but opposite results (34, 35). It has been argued that the discrepancy between these 2 trials may have been due to differences in vitamin B-12 doses (47). Although the higher dose of vitamin B-12 used in the Swiss Heart Study (34) might be associated with a stronger positive effect (6, 48), this would not explain the harm seen with B vitamin supplementation in the trial by Lange et al (35). At present, no evidence supports antioxidant or B vitamin supplements in post-PTCA patients.

Some limitations need to be considered when interpreting this meta-analysis. Trials using B vitamins and post-PTCA trials of antioxidants were small and therefore of limited reliability. Because only few such trials were available, even the pooled results had wide CIs. On the contrary, several trials of antioxidants in subjects not undergoing PTCA were at least moderately large. We are thus more confident in excluding a substantial benefit of antioxidant supplements on atherosclerosis progression.

Progression of atherosclerosis was evaluated by different imaging methods across studies. Measuring the progression of atherosclerosis is difficult, and these measures are subject to substantial random variability, to the possibility of between- and within-observer biases, and to temporal trends in measuring techniques. Furthermore, different methods have different levels of precision and measure slightly different aspects of atherosclerosis. Compared with measurements of intima-media thickness by B-mode ultrasound, coronary angiography assesses the lumen only and it lacks the sensitivity to detect early atherosclerosis (49, 50). Intravascular ultrasound evaluates the vessel wall and can be
used to measure the intima-media area (50). Quantitative coronary angiography and intravascular ultrasound, however, have a lower reproducibility than does B-mode ultrasound (50, 51), which likely adds to heterogeneity in our meta-analysis. To address the different methods used to evaluate atherosclerosis progression, we computed effect sizes to standardize the results of the trials to a uniform scale before combining the studies. Finally, few trials addressed the effect of losses to follow-up and some did not present the results based on the intention-to-treat principle. We expect that these methodologic limitations would tend to overestimate a potential benefit of the interventions and are unlikely to explain the lack of effect that we observed.

Vitamin-mineral supplements are widely used in the United States to prevent atherosclerosis and other chronic diseases (52). This use, however, is not supported by scientific evidence. Randomized trials assessing clinical endpoints indicate that β-carotene and vitamin E supplements may increase mortality, whereas B vitamin supplements do not prevent cardiovascular events. There is already enough evidence to recommend against using these supplements for cardiovascular disease prevention. Our meta-analysis showed that antioxidants do not prevent the development of atherosclerosis, thus providing a mechanistic explanation for the lack of effect of these supplements on clinical events. Furthermore, our meta-analysis found no evidence to support the use of vitamin-mineral supplements to prevent restenosis after PTCA. Although future research may identify a role for supplements in chronic disease prevention, antioxidants or B vitamins should not be used at present for cardiovascular disease prevention.

JB, ERM, and EG were responsible for the conception and design of the study, the critical revision of the article for important intellectual content, and the draft of the article. JB, ERM, RP-B, LJA, and EG were responsible for the analysis and interpretation of the data. JB, ERM, RP-B, LJA, and EG were responsible for the final approval of the article. JB, RP-B, and EG provided statistical expertise. JB and EG were responsible for the collection and assembly of the data. None of the authors declared a conflict of interest.

REFERENCES
31. DeMaio SJ, King SB, Lembo NJ, et al. Vitamin E supplementation,


