Antioxidant Vitamin Supplementation Reduces Arterial Stiffness in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials1–3

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Abstract

Background: Several studies tested the effects of supplementation with antioxidant vitamins on arterial stiffness, but the results were contradictory.

Objectives: The aim of our study was to conduct a systematic review and meta-analysis investigating the effect of antioxidant vitamins on arterial stiffness and to determine whether the effects on arterial stiffness vary according to dose, duration of intervention, and health or nutritional status of the included participants.

Methods: We searched 3 databases (Medline, Embase, and Scopus) for articles that potentially met the following eligibility criteria: 1) randomized controlled trials comparing antioxidant vitamins (vitamins C, E, and A and β-carotene) to either placebo or no active control in 2) adult participants aged ≥18 y; 3) antioxidant vitamins administered alone or in combination, irrespective of dose, duration, and route of administration; and 4) changes in arterial stiffness or arterial compliance. Data were pooled as standardized mean differences (SMDs) and analyzed using fixed- and random-effects models.

Results: Data synthesis showed that antioxidant vitamins reduced arterial stiffness significantly (SMD: −0.17; 95% CI: −0.26, −0.08; P < 0.001). This effect was significant in experimental (SMD: −1.02; 95% CI: −1.54, −0.49; P < 0.001) and primary prevention (SMD: −0.14; 95% CI: −0.24, −0.04; P < 0.01) studies, whereas a trend for reduced arterial stiffness was observed in studies including participants with diseases (SMD: −0.19; 95% CI: −0.40, 0.02; P = 0.08). Vitamin supplementation improved arterial stiffness irrespective of age group and duration of intervention. Antioxidant vitamins were more effective in participants with low baseline plasma concentrations of vitamins C (SMD: −0.35; 95% CI: −0.62, −0.07; P < 0.016) and E (SMD: −0.79; 95% CI: −1.23, −0.33; P < 0.01).

Conclusions: Supplementation with antioxidant vitamins has a small, protective effect on arterial stiffness. The effect may be augmented in those with lower baseline plasma vitamin E and C concentrations. This trial was registered at PROSPERO as CRD42014007260. J. Nutr. 144: 1594–1602, 2014.

Introduction

Arterial stiffening is a hallmark of aging and is closely associated with many pathologic conditions, including atherosclerosis, dyslipidemia, diabetes, and chronic kidney diseases (1). Reduction in arterial compliance (i.e., increased stiffness) leads to a faster reflection of the systolic wave from the peripheral small arteries to the heart, causing augmentation of central aortic pressure (2). This augmentation in central pressure leads to increased ventricular afterload and reduced coronary perfusion pressure, which eventually may cause myocardial hypertrophy, ischemia, and infarction (3). Thus, arterial stiffness is a precursor of cardiovascular disease (CVD)6 and is regarded as a marker for increased CVD risk and all-cause mortality (4).

Structural and functional changes in the vessel wall contribute to the onset and progression of arterial stiffness (5). Structural changes include the replacement of elastin with collagen and smooth-muscle proliferation (6). Oxidative stress and

References

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3 Supplemental Figures 1–3, Supplemental Tables 1 and 2, and Supplemental Methods are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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inflammation contribute significantly to this structural remodeling (7) through the process of smooth-muscle proliferation and collagen deposition (8), which increase the rigidity of vascular conduits and modify arterial compliance. Functional deterioration involves reduced bioavailability of NO and the onset of endothelial dysfunction (6). Specific dietary patterns, such as the Mediterranean diet or nutrients with anti-inflammatory and/or antioxidant properties, may have beneficial effects on vascular function (9). However, randomized controlled trials (RCTs) of the effects of antioxidant vitamin supplementation on arterial stiffness produced contradictory findings (10,11) so that the benefit, or otherwise, of such supplementation is uncertain.

The primary objective of our study was to investigate the effect of supplementation with antioxidant vitamins on arterial stiffness. The secondary objective was to determine whether the effects on arterial stiffness differed according to the type, dose, or duration of supplementation or the health or nutritional status of the included participants.

Methods

We conducted and report this systematic review according to the Cochrane and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (12,13).

Literature search. We searched 3 databases (Medline, Embase, and Scopus) for relevant articles from inception until December 2013. In addition, we conducted a manual search of the reference lists of the relevant articles and reviews to identify articles missed by the electronic search. The following MeSH terms and keywords were used: 1) antioxidants; 2) ascorbic acid; 3) vitamin A; 4) vitamin E; 5) β-carotene; 6) arterial compliance; 7) pulse wave; 8) vascular stiffness; and 9) applanation tonometry. Full details of our search criteria are reported in Supplemental Methods.

Study selection. Studies were eligible for inclusion in our systematic review if the following applied: 1) they were RCTs that compared antioxidant vitamins with placebo or no treatment control; 2) they involved adult participants aged ≥18 y; 3) they tested the antioxidant vitamins C, E, or A or β-carotene, administered alone or in combination, irrespective of the dose, duration, or route of administration or whether these vitamins were combined with other micronutrients or minerals; and 4) they reported changes in arterial stiffness or compliance irrespective of the instrument or the method of calculation [pulse-wave velocity (PWV), augmentation index (Alx), compliance coefficient, distensibility coefficient, or digital volume pulse]. No time or language restrictions were applied in searching the databases, and sample size was not an inclusion/exclusion criterion.

Two investigators (A.W.A. and C.O.) independently screened the titles and abstracts of the articles for eligibility for inclusion in the systematic review. If consensus was reached, the articles were excluded or moved to the next stage (full-text screening) as appropriate. If consensus was not reached, the article was moved to the next stage in which the full text of selected articles was evaluated to determine eligibility for inclusion in the systematic review. Disagreements were resolved by discussion between the reviewers until a consensus was reached.

Data extraction and quality assessment. The following information was extracted from the eligible articles: 1) authors, journal details, and year of publication; 2) participants (total number, male-to-female ratio, age, BMI, heart rate, systolic and diastolic blood pressure, health status, and use of prescribed drugs); 3) study characteristics (design, methods of randomization and blinding, and report of adverse effects); 4) nature of antioxidant intervention (type of antioxidant, dose, duration, route of administration, type of control, and any additional micronutrients); 5) arterial stiffness measurement (instrument, measurement site); and 6) circulating concentrations of antioxidant vitamins before and after intervention.

In addition, we adopted the modified Jadad score to assess the risk of bias of the included studies. Three main items related to randomization, blinding, and description of dropout or withdrawals were used in this scale to rate the quality of the included studies (14). Possible scores ranged from 0 to 5: a score of <3 indicates high risk of bias, a score of 3 indicates moderate risk of bias, and a score of ≥3 indicate low risk of bias (15).

Statistical analysis. The results from different measurements of arterial stiffness and compliance are reported on different scales, and, therefore, standardized mean differences (SMDs) were used as a summary statistic to allow comparison of effect sizes across studies. The SMD is estimated from the difference between the mean outcome values of the intervention and control groups divided by the pooled SD of the outcome values; this converts the estimated effect to SD units. SMDs of 0.2, 0.5, and 0.8 represent small, medium, and large effect sizes, respectively (16). Moreover, we implemented the use of the weighted mean difference (WMD) to pool data from studies that measured arterial stiffness outcome with the same unit (meters per second) for PWV and that used percentage when reporting the Alx. We undertook data synthesis, including calculation of effect sizes with 95% CIs, using both fixed- and random-effects models with inverse variance weighting (12). For graphical presentations of the arterial stiffness outcomes, forest plots were generated using the mean and SD at the end of the intervention (17). For studies that reported changes in arterial stiffness at ≥2 time points (e.g., acute and chronic effects of supplementation), the last measurement (chronic effect) was used in the meta-analysis. Data not provided in the main text or tables were extracted from the figures. For crossover trials, we used the mean and SD separately for the intervention and control conditions. This is regarded as a conservative approach that will reduce the power of these studies to show the true effect of the intervention (18). In trials with multiple treatment arms and a single control group, the sample size of the control group was divided by the number of treatment groups to avoid overinflation of the sample size (12). Statistical analyses were performed by usingSTATATA 12 (StataCorp).

Subgroup analyses were undertaken to investigate the variables that influenced the effects of supplementation on arterial stiffness. These factors included the following: 1) health status; 2) age; 3) vitamin doses; 4) duration of intervention; 5) baseline plasma concentration of vitamins; 6) study design; and 7) quality of the included studies. In the present meta-analysis, we used the definition used by the authors of the included studies for the reported health status of participants (healthy, at risk, or with underlying disease). Fixed-effects meta-regression analyses were used to determine whether participant mean baseline characteristics (BMI, heart rate, and systolic and diastolic blood pressure) influence the effect of antioxidant vitamin supplementation on arterial stiffness. Certain participants’ characteristics, such as alcohol and smoking history, lipoproteins, and cholesterol concentration, were not mentioned by most of the studies. Therefore, the above characteristics were not included in the subgroup analysis. Furthermore, we undertook sensitivity analyses to verify the effect of antioxidant vitamin supplementation on arterial stiffness by excluding studies with higher risk of bias on the Jadad score, studies lasting <2 wk, crossover studies, and studies with relatively small sample sizes.

Publication bias was evaluated by visual inspection of the funnel plot and by Egger’s regression test (19). Heterogeneity between studies was evaluated using Cochrane Q statistics; P > 0.1 indicates significant heterogeneity. The I² test was also used to evaluate consistency between studies in which a value <25% indicates low risk of heterogeneity, 25–75% indicates moderate risk of heterogeneity, and >75% indicates high risk of heterogeneity (20).

Results

Search results. A keyword search of the 3 databases yielded 456 articles after removal of duplicates. We retrieved the full text of 39 articles for additional assessment. Of these, 20 publications met our inclusion criteria and were included in the final analysis (Fig. 1).
Study characteristics. The total number of participants was 1909 (males: 1088; females: 821) with 8–1162 (median of 24) participants per study. The age of the participants ranged from 22 to 63.5 y (median of 29 y), and the duration of intervention varied from 1 d to 7 y (median of 56 d). Some of the studies (21–23) included independent subgroups that we investigated separately, resulting in a total of 26 subgroups in the final meta-analysis. Six studies (24–29) used crossover designs, 12 studies used parallel group designs (Table 1), and 1 study included both crossover and parallel subgroups (21). Eight of the included studies used vitamin C alone as the intervention agent (21,25,26,29–33), 7 studies used vitamin E alone (22,23,28,34–37), 2 studies combined vitamins C and E (24,27), 2 studies combined antioxidant vitamins with minerals (10,11), and 1 combined antioxidants with folic acid (38). No study tested vitamin A, although 1 study included b-carotene in a multi-micronutrient supplement (11). The dose of vitamin C ranged from 120 to 4000 mg (median of 2000 mg/d), and vitamin E dose ranged from 30 to 1000 IU (median of 360 IU/d).

Approximately half of the reports included in the systematic review came from studies involving healthy participants or chronic smokers (Table 1). For later subgroup comparison, we categorized these studies as primary prevention studies because the participants of these trials were free of underlying diseases or disorders. Three studies reported the recruitment of healthy participants who were exposed to insults to their endothelium with methionine (24,29) or glucose (26) and then challenged with antioxidant vitamin supplementation. We categorized these 3 studies as experimentally induced arterial stiffness. The remaining studies reported the recruitment of participants with underlying cardiometabolic disease, such as type 2 diabetes (2 studies) (30, 31), heart failure (2 studies) (21, 32), hypertension (27), coronary artery disease (10), or type 1 diabetes (36). We categorized these studies as secondary prevention studies. The methods used for measuring arterial stiffness differed between studies, but the most common methods were PWV and AIx (Table 1). Other measurements included elasticity coefficients (10,24,37,38), systemic arterial compliance (34–36), and peripheral arterial tonometry (30).

Qualitative analysis. The majority of the studies included in this meta-analysis were of moderate to high quality on the Jadad score (≥3), apart from 2 studies that appeared to have high risk of bias (10,24). Individually, more than half of the studies (12 studies) reported that antioxidant vitamin supplementation reduced arterial stiffness significantly (Table 1). Eight studies provided information on medication use (10,11,21,30–32,34,36), and 5 studies reported the dropout rate during the interventions (11,25,28,34,38). The washout period used in the crossover trials varied from 3 d to 4 wk, and 2 studies failed to report this information (21,29).

Meta-analysis. The meta-analysis demonstrated a significant reduction in arterial stiffness after antioxidant vitamin supplementation (SMD: -0.17; 95% CI: -0.26, -0.08; P < 0.001), with no significant heterogeneity among the included studies (X² = 27.4; P = 0.336; I² = 8.7%) (Fig. 2). Restricting the analysis to those studies using PWV (8 studies, 1453 participants) showed significant reductions in arterial stiffness (WMD: -0.20 m/s; 95% CI: -0.35, -0.06; P < 0.01) and the AIx (WMD: -4.71%; 95% CI: -6.18, -3.24; P < 0.001) with antioxidant vitamin supplementation (Figs. 3 and 4).
<table>
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<tr>
<th>Authors, year (reference)</th>
<th>Study design</th>
<th>Participants</th>
<th>Health status</th>
<th>Outcome</th>
<th>Age (^\text{a})</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
<th>Other vitamins</th>
<th>Duration</th>
<th>Control</th>
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<td>56 ± 2</td>
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<td>50 (34/16)</td>
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<td>55 ± 11</td>
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<td>38 (28/10)</td>
<td>Heart failure</td>
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<td>30 d</td>
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<td>PWV</td>
<td>64</td>
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<td>CO, DB</td>
<td>30 (30/0)</td>
<td>Hypertension</td>
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<td>36 (30/0)</td>
<td>Healthy</td>
<td>PWV, Ax</td>
<td>24 ± 0.4</td>
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<td>36 (30/0)</td>
<td>Healthy</td>
<td>PWV, Ax</td>
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<td>60 d</td>
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<td>Healthy</td>
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<td>400 IU</td>
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<td>70 d</td>
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<td>Healthy</td>
<td>CW</td>
<td>22 ± 2</td>
<td>1000</td>
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<td>70 (36/34)</td>
<td>Coronary heart disease</td>
<td>LAEI, SAEI</td>
<td>500</td>
<td>200 IU</td>
<td>60 mg coenzyme Q, 100 µg selenium</td>
<td>180 d</td>
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\(^{1}\) AIx, augmentation index; CC, compliance coefficient; CO, crossover; CW, Windkessel arterial compliance; DB, double-blind; DC, distensibility coefficient; LAEI, large arteries elasticity index; PWV, pulse-wave velocity; RH-PAT, reactive hyperemic peripheral arterial tonometry; SAC, systemic arterial compliance; SAEI, small arteries elasticity index; UB, non-blinded.

\(^{2}\) Values are means ± SDs, means, or ranges.

\(^{3}\) Yes, \(P < 0.05\); No, \(P \geq 0.05\).
Subgroup analysis. The type of supplementation revealed a significant reduction in arterial stiffness with vitamin E and with combined antioxidant vitamin supplementation, but the effect of vitamin C supplementation alone was not significant. Importantly, the analysis showed that supplementation reduced arterial stiffness in both experimental and primary prevention settings (Fig. 5). Although there was a trend toward reduced arterial stiffness in participants with diseases, the effect of supplementation in these secondary prevention trials was not statistically significant (SMD: -0.19; 95% CI: -0.40, 0.02; P = 0.08). Antioxidant vitamin supplementation was effective in reducing arterial stiffness in all age groups (22–63.5 y), but the effects were greater in

<table>
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<th>Author</th>
<th>SMD (95% CI)</th>
<th>% Weight (I-V)</th>
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<tr>
<td>Arcaro et al. [24]</td>
<td>-1.23 (-2.11, -0.35)</td>
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<td>I-V Overall (I-squared = 8.7%, p = 0.336)</td>
<td>-0.17 (-0.26, -0.08)</td>
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<tr>
<td>D-L Overall</td>
<td>-0.22 (-0.34, -0.11)</td>
<td></td>
</tr>
</tbody>
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FIGURE 2 Antioxidant vitamin supplementation (vitamins C, E, and A and β-carotene) reduced arterial stiffness in adults. The pooled estimates were obtained by using fixed-effects (I-V) and random-effects (D+L) models. Diamonds indicate the effect size of each study summarized as the SMD. The size of the shaded squares is proportional to the percentage weight of each study. Horizontal lines represent 95% CIs. The doses represent daily intake by each subgroup in that study. SMD, standardized mean difference.

FIGURE 3 Antioxidant vitamin supplementation (vitamins C, E, and A and β-carotene) reduced pulse-wave velocity (meters per second). The pooled estimates were obtained by using fixed-effects (I-V) and random-effects (D+L) models. Diamonds indicate the effect size of each study summarized as the WMD. The size of the shaded squares is proportional to the percentage weight of each study. Horizontal lines represent 95% CIs. The doses represent daily intake by each subgroup in that study. WMD, weighted mean difference.
younger (22–29 y) participants (SMD: −0.51; 95% CI: −0.76, −0.27; P < 0.001). Supplemental antioxidants improved arterial stiffness in acute (<2 wk), short-term (<3 mo), and longer-term (>3 mo) studies (Fig. 5).

In contrast to vitamin E, which was effective at all doses investigated (Fig. 5), vitamin C supplementation in high doses (4 g/d) did not reduce arterial stiffness (SMD: 0.07; 95% CI: −0.24, 0.37; P = 0.66). Greater improvements in arterial stiffness were
observed in participants with lower baseline plasma concentrations of vitamins C and E (Fig. 5). Meta-regression analysis (Supplemental Fig. 1) showed that the effect size was independent of baseline BMI ($\beta = 0.001; P = 0.97$), heart rate ($\beta = -0.04; P = 0.18$), and systolic ($\beta = 0.0007; P = 0.94$) or diastolic ($\beta = 0.001; P = 0.92$) blood pressure.

Sensitivity analysis. Removal of acute studies (≤2 wk), including experimental studies, did not change the significance of our finding (SMD: $-0.17; 95\% CI: -0.26, -0.08; P < 0.001$). In addition, excluding studies with low Jadad scores (≤2) did not affect the results substantially (SMD: $-0.15; 95\% CI: -0.24, -0.06; P = 0.001$). Similarly, although omitting crossover studies reduced the effect size, it remained statistically significant (SMD: $-0.14; 95\% CI: -0.24, -0.05; P = 0.003$). Furthermore, Supplemental Table 2 included subgroup analyses using random-effects meta-analysis.

Publication bias. The funnel plot showed asymmetry in the distribution of the studies included in the meta-analysis, which may denote either publication bias or small-study effect (the tendency of small studies to show higher effect sizes than larger studies) (39). Therefore, we conducted a sensitivity analysis excluding studies with relatively small sample sizes (<20 participants). This restricted analysis (14 studies, 1792 participants) showed that antioxidant vitamin supplementation reduced arterial stiffness significantly (SMD: $-0.14; 95\% CI: -0.24, -0.05; P = 0.002$) and provided no significant evidence of publication bias (Egger's regression test, $\beta = -0.62; P = 0.10$) (Supplemental Figs. 2 and 3).

Discussion

Our meta-analysis revealed that supplementation with antioxidant vitamins produces a small but significant reduction in arterial stiffness. The effectiveness of supplementation appeared to be blunted in those at greater risk of vascular impairment because the effect size was greater in healthy participants than in those with cardiometabolic disease. Furthermore, antioxidant vitamin supplementation showed greater improvement of arterial stiffness indices in younger participants. This suggests that the presence of more advanced structural and functional alterations of the vascular tree (arteriosclerosis) may lessen the benefits of antioxidant vitamins. Furthermore, antioxidant vitamin supplementation was considerably more effective in improving arterial stiffness in participants with lower baseline plasma concentrations of vitamins C and E.

The beneficial effects of antioxidant vitamins on vascular stiffness may be explained by the reduction of the damaging effects of free radicals on structural and functional components of the vessel walls (27). Antioxidant vitamins inactivate free radicals, reduce inflammation, and therefore protect the integrity of the vascular wall (40). Furthermore, antioxidant vitamins increase the bioavailability of the vasodilator and anti-inflammatory molecule NO (6). However, our finding that the response in arterial stiffness to supplementation differs for each particular vitamin may suggest that each vitamin has specific effects on the vascular wall, beyond any generic antioxidant effect. These effects may include the pleiotropic effects of vitamin A through its role in regulating gene expression or a role of vitamin C in structural remodeling of the vessel wall by modulating the collagen–elastin balance (41,42). The administration of vitamin C alone was ineffective in improving arterial stiffness, which may be explained by the ineffectiveness of the large doses of ascorbic acid (2–4 g/d) used in such studies (Table 1). Such doses are many folds greater than the nutritional needs for vitamin C and may not be advantageous. It was suggested that, when used in high doses, vitamin C may have a pro-oxidant effect (43) that may deteriorate rather than ameliorate arterial stiffness (44). However, these findings were challenged by others (45–47). In addition, vitamin C is a cofactor in the biosynthesis of collagen and in large doses may enhance collagen deposition in the vessel wall (41). Such effects would be expected to lower vascular elasticity, but whether this occurs in nonpathologic tissue is not known.

Despite the supporting evidence from observational studies on the beneficial effects of antioxidant vitamins on cardiovascular health, the results from RCTs testing the efficacy of antioxidant vitamins on CVD risk were discouraging (48–50). Furthermore, these trials linked supplemental vitamin E with an increased risk of cardiovascular events and mortality (51,52). In addition to their undoubted strengths, these large RCTs have several limitations, including the potential for masking beneficial effects in particular subgroups of the population. For example, subgroup analysis revealed the beneficial effects of antioxidant vitamin supplementation on cardiovascular outcomes in individuals with low baseline plasma concentrations of vitamins in some trials (53,54). Our findings of larger effect sizes in subpopulations with lower baseline plasma concentrations of vitamins C and E are in line with these observations.

However, the evidence available for inclusion in the present meta-analysis had a number of limitations that may have implications for the potential public health impact of our findings. First, the majority of the included studies have a small sample size and are characterized by a moderate quality on the Jadad score. More, larger, and better-quality studies would increase confidence in the findings from the present analysis. Second, PWV, the AIx, and arterial compliance are surrogate CVD endpoints, and, therefore, changes in arterial stiffness may not necessarily reflect changes in progression toward the more severe functional impairments and organ damage that precipitate major cardiovascular events. However, a recently published meta-analysis of data from 16 prospective studies involving 17,635 participants demonstrated that a 1 m/s increase in aortic PWV was associated with a 7% increased risk of subsequent cardiovascular events (55). The mean reduction in PWV after antioxidant vitamin supplementation observed in the present analysis was 0.2 m/s (Fig. 3), which highlights the likely modest effect of such supplementation on CVD risk. Furthermore, Vlachopoulos et al. (36) showed that a 10% increase in the AIx is associated with 31.8% increased risk of cardiac events, which compares with a mean reduction of 4.7% in the AIx after antioxidant vitamin supplementation in the present analysis (Fig. 4). Third, the observed asymmetrical funnel plot for all studies (Supplemental Fig. 2) denoted the presence of publication bias or a small-studies effect (39). However, when we excluded small studies, i.e., those with <20 participants, antioxidant vitamin supplementation in the remaining larger studies showed significant improvement in arterial stiffness, and the test for publication bias was nonsignificant (Supplemental Fig. 3). This provides some reassurance that the observed benefit of supplementation is robust across studies of different sizes. Fourth, because of the lack of a viable alternative, we accepted the definition used by the authors of included studies when describing the health status of participants. Because of the lack of use of objective and purposeful measures for characterizing participants as “healthy,” it is possible that such groups of participants are relatively heterogeneous. Last, our primary method for summarizing the effect size attributable to antioxidant vitamin supplementation
across studies was the SMD. Some may argue that this method of calculating the effect size might be less clinically meaningful and that it may be more difficult for some readers to interpret results presented in this way (16). However, others may argue that this is the best method to facilitate the meta-analysis of results from the largest number of (relevant) studies and to avoid the potential for bias that would follow from omitting studies that met the inclusion criteria but presented results for the key outcome in some nonstandard measure (57). We argue the merits of an inclusive approach.

The results of this meta-analysis demonstrate that supplementation with antioxidant vitamins reduces arterial stiffness, but the effect sizes are modest. The vascular protection afforded by such supplementation is enhanced in healthy, young individuals and in individuals with lower circulating concentrations of vitamins C and E. However, the potential public health importance of these findings remains to be tested in suitably designed personalized (or stratified) intervention studies.

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