Selenium and coronary heart disease: a meta-analysis 1–3

Gemma Flores-Mateo, Ana Navas-Acien, Roberto Pastor-Barriuso, and Eliseo Guallar

ABSTRACT

Background: It is hypothesized that low selenium concentrations are associated with an increased risk of cardiovascular disease and that selenium supplements prevent coronary heart disease.

Objective: The objective was to perform a meta-analysis on the association of selenium biomarkers with coronary heart disease endpoints in observational studies and on the efficacy of selenium supplements in preventing coronary heart disease endpoints in randomized trials.

Design: The MEDLINE and the Cochrane Library databases were searched for studies conducted from 1966 through 2005. Relative risks were pooled by using an inverse-variance weighted random-effects model.

Results: Twenty-five observational studies (14 cohort and 11 case-control studies) that measured blood or toenail selenium concentrations and 6 randomized trials that evaluated supplements containing selenium met our inclusion criteria. The pooled relative risk in a comparison of the highest with the lowest selenium concentration categories was 0.85 (95% CI: 0.74, 0.99) in cohort studies and 0.43 (0.29, 0.66) in case-control studies. In observational studies, a 50% increase in selenium concentrations was associated with a 24% (7%, 38%) reduction in coronary heart disease risk. In randomized trials, the pooled relative risk in a comparison of supplements containing selenium with placebo was 0.89 (0.68, 1.17).

Conclusions: Selenium concentrations were inversely associated with coronary heart disease risk in observational studies. Because observational studies have provided misleading evidence for other antioxidants, the validity of this association is uncertain. Few randomized trials have addressed the cardiovascular efficacy of selenium supplementation, and their findings are still inconclusive. Evidence from large ongoing trials is needed to establish low selenium concentrations as a cardiovascular disease risk factor. Currently, selenium supplements should not be recommended for cardiovascular disease prevention. Am J Clin Nutr 2006;84:762–73.

KEY WORDS Selenium, coronary heart disease, atherosclerosis, meta-analysis, systematic review

INTRODUCTION

Selenium is an essential trace mineral involved in protection against oxidative damage via selenium-dependent glutathione peroxidases and other selenoproteins (1). Current recommendations on dietary intake of selenium are based on optimizing the activity of plasma glutathione peroxidases (2). The recommended dietary allowance for selenium that is estimated to be sufficient to meet the nutritional needs of nearly all healthy adults is 55 μg/d (2, 3). Plant foods, meat, and seafood are the major dietary sources of selenium, predominantly as selenomethionine and selenocysteine, but the selenium content of foods varies geographically depending on soil and water concentrations and use of selenium-containing fertilizers (4–8). For this reason, dietary assessment methods are inappropriate for estimating selenium exposure (6) and observational studies of selenium status are based on biomarkers such as toenail, blood, erythrocyte, or serum and plasma selenium concentrations (7–9).

Because of its antioxidant properties, it has long been hypothesized that selenium may prevent cardiovascular and other chronic diseases. Selenium supplementation increases enzymatic antioxidant activity (10–12) and decreases lipid peroxidation (12–14). The effect of selenium on atherosclerotic cardiovascular disease, however, is uncertain. Observational studies (15–28) investigating the association of low selenium concentrations with cardiovascular outcomes and randomized trials (14, 29–33) investigating whether selenium supplements prevent coronary heart disease have been inconclusive, but the evidence has not been appraised systematically.

The objective of the present meta-analysis was to synthesize results from observational studies of the association of selenium biomarkers with coronary heart disease endpoints and from results of clinical trials of the efficacy of selenium supplements in preventing coronary heart disease endpoints.

METHODS

We searched MEDLINE for observational studies and randomized trials investigating the relation of selenium with coronary heart disease. We used free text and the Medical Subject Headings (MeSH) terms “selenium,” “selenite,” “selenate,”

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“cardiovascular disease,” “Khesan disease,” “myocardial infarction,” “stroke,” “peripheral arterial disease,” and “mortality.” The search period was January 1966 through March 2006; no language restrictions were added. We also searched the Cochrane Central Register of Controlled Trials and reviewed the reference lists of relevant original papers and review articles.

We aimed to identify all observational studies that assessed the association of selenium concentrations in blood or toenails with clinical coronary heart disease outcomes and all randomized trials that assessed the efficacy of selenium supplements, either alone or in combination with other vitamins or minerals, for preventing coronary heart disease (Figure 1). Our exclusion criteria were the following: 1) no original research (reviews, editorials, nonresearch letters); 2) studies not conducted in humans; 3) case reports or case series; 4) ecologic studies; 5) lack of data on selenium exposure; 6) studies of angiographically defined endpoints or of angina pectoris as the endpoint; 7) studies of other cardiovascular outcomes such as heart failure, stroke, peripheral arterial disease, or nonatherosclerotic heart disease; and 8) observational studies conducted in populations of patients with coronary heart disease at baseline. We additionally excluded a small autopsy-based study (21 case and 22 control subjects) that did not measure any of the standard selenium biomarkers (34). For populations originating several reports, the publication with the longest follow-up was selected (26, 33, 35).

Two investigators (GF-M and AN-A) independently reviewed search results and selected articles to determine eligibility and to abstract study data. They resolved discrepancies by consensus. The investigators of the original studies were contacted if relevant information on eligibility or key study data were not available in the published report. For observational studies, the criteria used by Longnecker et al (36) were adapted to assess study quality (Appendix A). For randomized trials, we used the quality criteria of Jadad et al (37).

The a priori selected endpoint was coronary heart disease, which was defined as any combination of fatal or nonfatal coronary heart disease and myocardial infarction. Studies reporting only total cardiovascular endpoints were also included, because coronary heart disease is the major contributor to cardiovascular disease in many populations.

**Statistical analysis**

Observational studies and randomized trials were analyzed separately. For observational studies, measures of association (odds ratios, relative risks, or hazard ratios) and their 95% CIs were abstracted or derived by using data reported in the publications. When several measures of association were reported, we selected the measure obtained from the model with the highest number of categories for selenium exposure first and the measure adjusted for most covariates second. For studies that categorized selenium exposure, we compared the risk of coronary heart disease in the highest with the lowest selenium category. For one study that analyzed selenium only as a continuous variable (25), we derived the relative risk associated with an increase of one SD in selenium concentrations in noncase subjects. For studies reporting only mean selenium concentrations in case and noncase subjects (16, 28, 38-47), we used linear discriminant function methods (48) to calculate the relative risk in a comparison of the 75th to the 25th percentiles of the selenium distribution in noncase subjects, assuming a normal distribution for selenium.

To pool relative risk estimates from individual studies, we used an inverse-variance weighted random-effects model. Heterogeneity was quantified with the $I^2$ statistic (49), which describes the proportion of total variation in study estimates due to...
TABLE 1
Prospective cohort studies of selenium and coronary heart disease (CHD)

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Country</th>
<th>Population</th>
<th>Men</th>
<th>Mean age</th>
<th>Endpoint ascertainment</th>
<th>Follow-up</th>
<th>Outcome</th>
<th>No. of case subjects</th>
<th>Selenium concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salonen, 1982 (15)</td>
<td>Finland</td>
<td>General population Eastern Finland</td>
<td>73</td>
<td>50</td>
<td>Hospital records, death certificate</td>
<td>7</td>
<td>CHD mortality</td>
<td>95/95</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Miettinen, 1983 (16)</td>
<td>Finland</td>
<td>Men with high CVD risk</td>
<td>100</td>
<td>48</td>
<td>Chest pain, cardiac enzyme, ECG</td>
<td>5–7</td>
<td>AMI incidence</td>
<td>33/64</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Virtamo, 1985 (17)</td>
<td>Finland</td>
<td>Rural men</td>
<td>100</td>
<td>55–74</td>
<td>Clinical exam, death certificate, ECG</td>
<td>5</td>
<td>CHD mortality</td>
<td>30/591</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Salonen, 1985 (18)</td>
<td>Finland</td>
<td>Eastern Finland Heart Survey</td>
<td>75</td>
<td>54</td>
<td>Death certificate</td>
<td>5</td>
<td>CHD mortality</td>
<td>92/92</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Ringstad, 1986 (19)</td>
<td>Norway</td>
<td>First Tromso Heart Study</td>
<td>100</td>
<td>20–49</td>
<td>Death certificate or chest pain, enzyme, ECG</td>
<td>8</td>
<td>AMI incidence</td>
<td>99/99</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Kok, 1987 (20)</td>
<td>Netherlands</td>
<td>General population</td>
<td>56</td>
<td>67</td>
<td>Death certificate</td>
<td>9</td>
<td>CVD mortality</td>
<td>84/168</td>
<td>Serum (NAA)</td>
</tr>
<tr>
<td>Ringstad, 1987 (21)</td>
<td>Norway</td>
<td>Second Tromso Heart Study</td>
<td>100</td>
<td>46</td>
<td>Hospital records, death certificates</td>
<td>6</td>
<td>AMI incidence</td>
<td>59/59</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Suadicani 1992 (22)</td>
<td>Denmark</td>
<td>Copenhagen Male Study</td>
<td>100</td>
<td>63</td>
<td>Hospital records, death certificates</td>
<td>3</td>
<td>CHD incidence</td>
<td>107/2715</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Salvini, 1995 (23)</td>
<td>USA</td>
<td>Physicians' Health Study</td>
<td>100</td>
<td>40–84</td>
<td>Questionnaires, hospital records, death certificates</td>
<td>5</td>
<td>AMI incidence</td>
<td>186/186</td>
<td>Serum (NAA)</td>
</tr>
<tr>
<td>Mammiemi, 1998 (24)</td>
<td>Finland</td>
<td>General elderly population</td>
<td>53</td>
<td>≥65</td>
<td>Death certificates</td>
<td>13</td>
<td>CVD mortality</td>
<td>142/202</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Klander, 2001 (25)</td>
<td>Sweden</td>
<td>Men born in Uppsala in 1920–1924</td>
<td>100</td>
<td>50</td>
<td>Death certificate</td>
<td>25</td>
<td>CVD mortality</td>
<td>301/1727</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Yoshizawa, 2003 (26)</td>
<td>USA</td>
<td>Health Professionals Follow-Up Study</td>
<td>100</td>
<td>62</td>
<td>Questionnaires, medical records, death certificates</td>
<td>5</td>
<td>CHD incidence</td>
<td>470/465</td>
<td>Toenails (NAA)</td>
</tr>
<tr>
<td>Wei, 2004 (27)</td>
<td>China</td>
<td>General population trial of Linxian</td>
<td>55</td>
<td>57</td>
<td>Monthly follow-up</td>
<td>15</td>
<td>CHD mortality</td>
<td>116987</td>
<td>Serum (AAS)</td>
</tr>
<tr>
<td>Akbaraly, 2005 (28)</td>
<td>France</td>
<td>Etude du Vieillissement Arteriel (EVA)</td>
<td>41</td>
<td>65</td>
<td>Death certificate, Hospital records</td>
<td>9</td>
<td>CVD mortality</td>
<td>22/1367</td>
<td>Serum (AAS)</td>
</tr>
</tbody>
</table>

<sup>1</sup> AAS, atomic absorption spectroscopy; ECG, electrocardiogram; AMI, acute myocardial in fraction; NR, not reported; CVD, cardiovascular disease; NAA, neutron activation analysis.

<sup>2</sup> x ± SD (all such values).

<sup>3</sup> In µg/g.
heterogeneity. We used meta-regression to evaluate whether results were different by selenium concentrations in the reference category (> or <70 μg selenium/L), study design (cohort compared with case-control), selenium biomarker (serum compared with other), outcome (mortality only compared with mortality or morbidity outcomes), or country (European compared with other). Because study design was the only significant determinant of heterogeneity, we separated the analyses for prospective cohort and case-control studies.

For observational studies that reported ≥3 categories of exposure, we additionally conducted a random-effects dose-response meta-analysis using the methods of Greenland and Longnecker (50). Because selenium concentrations in the reference categories differed across studies, study-specific results were pooled in terms of relative changes in selenium concentrations with respect to the reference category. We evaluated departures from the linear trend by testing for a quadratic term in the dose-response meta-analysis (50).

Clinical trials were analyzed according to the intention-to-treat principle. We computed relative risks and 95% CIs of coronary heart disease in a comparison of participants assigned to supplements containing selenium with those assigned to control supplements. We used an inverse-variance weighted random-effects model to pool relative risk estimates.

For both observational studies and clinical trials, we assessed the relative influence of each study on pooled estimates by omitting one study at a time. Finally, we assessed publication bias using funnel plots (51). Statistical analyses were conducted with Stata version 8 (STATA Corp, College Station, TX) and with S-PLUS version 7 (Insightful Corporation, Seattle, WA).

RESULTS

Meta-analysis of observational studies

Fourteen prospective cohort studies (15–28) (Table 1) and 11 case-control studies (38, 40–47, 52, 53) (Table 2) met our inclusion criteria (Figure 1). The studies were published between 1982 and 2005. Most studies, except 4 (23, 26, 27, 52), were performed in Europe. The number of case subjects varied between 22 (28) and 683 (53). One cohort study (28) and 7 case-control studies (38, 40, 43–47) did not control for potential confounders. Cohort studies tended to fulfill prespecified quality criteria, whereas case-control studies varied widely (Appendix 1).

Except for 3 cohort (21, 23, 24) and 2 case-control (44, 47) studies, most studies found an inverse association of selenium with the risk of coronary heart disease (Figure 2). The pooled relative risk in a comparison of the highest to the lowest category of selenium concentration was 0.85 in cohort studies (95% CI: 0.74, 0.99; P for heterogeneity = 0.33; F² = 5%) and 0.43 in case-control studies (95% CI: 0.29, 0.66; P for heterogeneity < 0.001; F² = 88%). Other sources of heterogeneity investigated, including the influence of selenium concentrations of the reference category, were minor and not statistically significant. Specifically, we used a meta-regression model to evaluate whether the relative risk of coronary heart disease in a comparison of the highest and lowest categories of selenium exposure were similar in studies with plasma or serum selenium concentrations in the reference category > or <70 μg/L. The relative risks in both types of studies were similar and the difference was not statistically significant (difference in log relative risk: 0.07; 95% CI: −0.51, 0.64; P = 0.82).

In sensitivity analyses, exclusion of individual studies did not modify the estimates substantially, with pooled relative risks ranging from 0.78 to 0.90 in cohort studies and from 0.41 to 0.59 in case-control studies. Funnel plots did not suggest the presence of publication or related biases (not shown).

For studies with ≥3 selenium categories, the dose-response meta-analysis showed a decreasing trend of coronary heart disease risk with increasing selenium concentrations (Figure 3). The pooled relative risk associated with a 50% increase in selenium concentrations was 0.76 (95% CI: 0.62, 0.93; P for heterogeneity = 0.06). Adding a quadratic term to the model did not significantly improve model fit (P = 0.64).

Meta-analysis of randomized trials

Six trials (14, 29–33), published between 1989 and 2004, met our inclusion criteria (Table 3). These trials randomly assigned a total of 17 766 participants. Four trials used selenium combined with other vitamins or minerals (14, 30, 32, 54), and 2 trials used selenium alone (29, 33). Selenium doses were 75 μg/d (54), 100 μg/d (14, 29, 30, 32), or 200 μg/d (33). Only one trial used selenite (30), whereas 3 trials used selenium yeast (29, 32, 33). In 2 trials, the form of selenium was not specified. All trials were placebo-controlled, and all except one (30) were double-blinded. The length of follow-up ranged from 0.5 to 7.6 y.

The pooled relative risk in a comparison of selenium supplementation to placebo across all trials was 0.89 (95% CI: 0.68, 1.17; P for heterogeneity = 0.22; F² = 40%) (Figure 4). Exclusion of any individual trial did not substantially change the overall pooled relative risk estimates, which ranged from 0.63 to 0.92.

DISCUSSION

In the present meta-analysis, we identified a moderate but statistically significant inverse association between selenium concentrations in several tissues and coronary heart disease outcomes in observational studies. A 50% increase in selenium concentrations was associated with a 24% reduced risk of coronary events. The validity of this association, however, is uncertain, because observational studies have been unreliable in determining the cardiovascular effects of other antioxidants and vitamins, such as β-carotene, vitamin E, and folate (55). Few randomized controlled trials have addressed the effect of selenium supplementation on clinical endpoints. In these trials, participants taking supplements containing selenium had a nonsignificant 11% reduction in coronary events, but the trials were small and selenium was given in combination with other vitamins or minerals in all but 2 trials. Overall, the evidence is still inadequate to establish a protective role of selenium in coronary heart disease.

Biological plausibility

Selenium, a constituent of selenoproteins as selenocysteine, has important antioxidant properties (1, 56, 57). Selenoproteins with antioxidant functions include glutathione peroxidases, which reduce hydrogen peroxide and lipid and phospholipid hydroperoxides; thioredoxin reductases, which help regenerate antioxidant systems and maintain the intracellular redox status (1); and selenoprotein P, which may protect endothelial cells...
<table>
<thead>
<tr>
<th>Study, year</th>
<th>Country</th>
<th>Percentage of men among control subjects</th>
<th>Mean age of control subjects</th>
<th>Type of control subjects</th>
<th>Source of case subjects</th>
<th>Outcomes</th>
<th>No. of case subjects/control subjects</th>
<th>Selenium assessment (technique)</th>
<th>Selenium concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oster, 1986 (38)</td>
<td>Germany</td>
<td>100</td>
<td>52</td>
<td>University employees</td>
<td>University health care center Hospital</td>
<td>AMI incidence</td>
<td>49/41</td>
<td>Serum (AAS)</td>
<td>56.0 ± 15.0&lt;sup&gt;7&lt;/sup&gt; ± 8.0 ± 11.0</td>
</tr>
<tr>
<td>Auzepy, 1987 (40)</td>
<td>France</td>
<td>60</td>
<td>34</td>
<td>Nursing and medical staff</td>
<td>Hospital</td>
<td>AMI incidence</td>
<td>31/48</td>
<td>Serum (AAS)</td>
<td>73.6 ± 13.0 ± 84.7 ± 12.4</td>
</tr>
<tr>
<td>Salonen, 1988 (41)</td>
<td>Finland</td>
<td>100</td>
<td>54</td>
<td>Kuopio Ischemic Heart Disease Study</td>
<td>Kuopio Ischemic Heart Disease Study Hospital</td>
<td>CHD prevalence</td>
<td>175/449</td>
<td>Serum (AAS)</td>
<td>81.5 ± 19.2 ± 85.0 ± 21.6</td>
</tr>
<tr>
<td>Kok, 1989 (42)</td>
<td>Netherlands</td>
<td>70</td>
<td>59</td>
<td>General population</td>
<td>Hospital</td>
<td>AMI incidence</td>
<td>84/84</td>
<td>Plasma</td>
<td>100.8 ± 27.5 ± 106.8 ± 23.8</td>
</tr>
<tr>
<td>Beaglehole, 1990 (52)</td>
<td>New Zealand</td>
<td>60</td>
<td>52</td>
<td>General population</td>
<td>Monica Project Registry</td>
<td>AMI incidence</td>
<td>125/128</td>
<td>Serum (AAS)</td>
<td>58.2 ± 13.0 ± 82.7 ± 20.7</td>
</tr>
<tr>
<td>Thiele, 1995 (43)</td>
<td>Germany</td>
<td>NR</td>
<td>NR</td>
<td>Healthy blood donors</td>
<td>Hospital</td>
<td>AMI incidence</td>
<td>83/82</td>
<td>Serum (AAS)</td>
<td>71.0 ± 13.4 ± 78.9 ± 13.4</td>
</tr>
<tr>
<td>Kardinaal, 1997 (53)</td>
<td>8 European countries and Israel</td>
<td>100</td>
<td>53</td>
<td>General population and clinic based</td>
<td>Coronary unit</td>
<td>AMI incidence</td>
<td>683/729</td>
<td>Whole blood</td>
<td>86.8 ± 15.8 ± 105.8 ± 13.4</td>
</tr>
<tr>
<td>Coudray, 1997 (44)</td>
<td>France</td>
<td>40</td>
<td>65</td>
<td>General population</td>
<td>Surveys</td>
<td>AMI prevalence</td>
<td>50/498</td>
<td>Plasma (AAS)</td>
<td>88.4 ± 16.6 ± 85.2 ± 15.0</td>
</tr>
<tr>
<td>Navarro-Alarcon, 1999 (45)</td>
<td>Spain</td>
<td>NR</td>
<td>NR</td>
<td>General population</td>
<td>Hospital</td>
<td>CHD prevalence</td>
<td>50/130</td>
<td>Serum (AAS)</td>
<td>55.5 ± 16.7 ± 74.9 ± 27.3</td>
</tr>
<tr>
<td>Bor, 1999 (45)</td>
<td>Turkey</td>
<td>83</td>
<td>51</td>
<td>NR</td>
<td>Emergency room</td>
<td>AMI incidence</td>
<td>27/24</td>
<td>Plasma</td>
<td>63.7 ± 12 ± 82.2 ± 14.6</td>
</tr>
<tr>
<td>Zachara, 2001 (47)</td>
<td>Poland</td>
<td>62</td>
<td>57</td>
<td>NR</td>
<td>Coronary unit</td>
<td>AMI incidence</td>
<td>49/58</td>
<td>Whole blood</td>
<td>71.4 ± 18.2 ± 73.1 ± 18.0</td>
</tr>
</tbody>
</table>

1. AMI, acute myocardial infarction; AAS, atomic absorption spectroscopy; NAA, neutron activation analysis.
2. Measured in μg/L, unless otherwise specified.
3. x ± SD (all such values).
4. Measured in μg/g hemoglobin.
5. Measured in μg/g.
6. Median (25th and 75th percentiles).
against peroxynitrite and lipid peroxidation (58, 59). In selenium-deficient humans, selenium supplementation increases enzymatic antioxidant activity (10–12, 60) and decreases lipid peroxidation (12–14). In addition, selenium may reduce the production of inflammatory prostaglandins and leukotrienes by neutralizing peroxide intermediates (1).

Low selenium concentrations may also increase cardiovascular disease risk through other mechanisms. By shifting prostaglandin synthesis from prostacyclin to thromboxane, low selenium may increase platelet aggregability and vasoconstriction (1, 56, 61). Randomized trials of selenium supplementation on platelet function, blood pressure levels, and lipid profile, however, have been contradictory (12, 14, 62, 63). Finally, selenium may protect the cardiovascular system from toxic metals that have been implicated in atherogenesis, such as mercury, cadmium, and arsenic, by preventing metal-induced oxidative damage or by forming inactive complexes with metals (56, 64, 65).

Selenium supplementation decreased the incidence of Keshan disease, a congestive cardiomyopathy that mostly affects children and young women in some selenium-poor areas of China (1, 66). However, whether selenium deficiency results in increased atherosclerosis is unclear (1, 56, 67).

**Low selenium concentration as a cardiovascular disease risk factor**

Biomarkers of selenium, such as toenail, blood, erythrocyte, and whole blood, have all been shown to reflect selenium exposure (7, 8). However, the interpretation of biomarkers is complex because selenium concentrations depend not only on exposure, but also on the form of selenium intake, on selenium metabolism, and on pathophysiological responses to conditions associated with increased oxidative stress or inflammation. Consequently, although selenium concentrations are correlated with intake, the comparability of

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**FIGURE 2.** Meta-analysis of the association of selenium with coronary heart disease in observational studies. Studies are divided by study design (cohort or case-control) and by selenium biomarker (serum, toenail, erythrocyte, or whole blood). Relative risks (RRs) correspond to comparisons of extreme categories of exposure within each study. The area of each square is proportional to the inverse of the variance of the log RR. Horizontal lines represent 95% CIs. Diamonds represent pooled estimates from inverse-variance weighted random-effects models. For case-control studies with multiple biomarkers, we used the biomarker with the longest half-life (toenail > whole blood and erythrocyte > serum) to measure the overall RR. Ca, case subjects; NC, noncase subjects; DM, diabetes mellitus; HT, hypertension; SES, socioeconomic status; Hb, hemoglobin. ■ Indicates categories that were adjusted for; □ indicates categories that were not adjusted for.
different biomarker concentrations observed in different studies is uncertain. In addition, selenium in blood and other tissues is present as selenocysteine in selenoproteins, which are maximized at plasma selenium concentrations between 70 and 90 μg/L, and as selenomethionine in proteins that contain methionine, with no apparent maximum concentration (68, 69). As a result, high selenium concentrations may reflect selenomethionine incorporated nonspecifically in proteins instead of methionine and may thus be considered primarily a marker of high dietary intake of plant-derived foods grown in selenium-rich soils. None of the observational studies included in the present review provided information on the selenium content of plant-derived foods or other food items. In addition, selenomethionine and selenium yeast supplements also increase selenomethionine concentrations without increasing selenoprotein activity in populations with adequate selenium intakes (70). In most observational studies included in this meta-analysis, serum and whole-blood selenium concentrations in the highest category of exposure were >80 μg selenium/L. In some studies, the cutoff for the reference category was also >80 μg selenium/L (19–21, 23, 42, 43).

The prospective cohort studies summarized in the present meta-analysis show, in the aggregate, a moderate inverse association between selenium concentrations and coronary heart disease endpoints. This inverse association appeared to be linear throughout the range of selenium concentrations and was observed in populations from different countries with different baseline selenium concentrations. In our dose-response meta-analysis, we estimated that a 50% increase in selenium concentrations was associated with a 24% decreased risk of coronary heart disease. In trials, a dose of 100 μg selenium/d increased blood selenium from 82 to 122 μg/L (a 49% increase) (29), whereas a dose of 200 μg/d increased blood selenium from 67 to 190 μg/L (a 184% increase) (71). Most trials in our meta-analysis used doses of ≥100 μg selenium/d, yet the overall reduction in coronary heart disease was only 11%. Thus, observational studies may also overestimate the association between selenium and coronary heart disease.

The different characteristics of subjects receiving high and low selenium diets or selenium supplements, factors affecting selenium concentrations, residual confounding by socioeconomic status, education, or other cardiovascular risk factors, and selective publication of studies that show an inverse association could contribute to create the inverse association observed between selenium concentrations and coronary heart disease. A better understanding of the determinants of selenium intake and selenium concentrations is needed before low selenium concentrations can be established as a cardiovascular risk factor on the basis of observational evidence.

Is the use of selenium supplements justified for cardiovascular disease prevention?

The difficulties in interpreting the findings of observational studies of antioxidants and coronary endpoints highlight the need for better understanding of the determinants of selenium intake and selenium concentrations to establish low selenium concentrations as a cardiovascular risk factor.
**TABLE 3**
Randomized trials of selenium supplementation and risk of coronary heart disease (CHD) 1

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Country</th>
<th>Population</th>
<th>Men</th>
<th>Mean age</th>
<th>Selenium form (dose µg/d)</th>
<th>Selenium combined with other vitamins or minerals</th>
<th>Factorial design (factorial intervention)</th>
<th>Placebo-controlled</th>
<th>Double-blind</th>
<th>Follow-up</th>
<th>Outcomes</th>
<th>Quality score 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korpela, 1989 (29)</td>
<td>Finland</td>
<td>Patients with AMI</td>
<td>77</td>
<td>57</td>
<td>Selenium yeast (100)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>0.5</td>
<td>CHD incidence</td>
<td>2</td>
</tr>
<tr>
<td>Kuklinski, 1994 (30)</td>
<td>Germany</td>
<td>Patients with AMI</td>
<td>NR</td>
<td>NR</td>
<td>Sodium selenite (100)</td>
<td>Yes (100 mg coenzyme Q10, 15 mg Zn, 1 mg vitamin A, 2 mg vitamin B-6, 90 mg vitamin C, 15 mg vitamin E)</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>1.0</td>
<td>AMI mortality</td>
<td>1</td>
</tr>
<tr>
<td>Brown, 2001 (14)</td>
<td>Canada and USA</td>
<td>Patients with CHD</td>
<td>87</td>
<td>53</td>
<td>Selenium yeast (100)</td>
<td>Yes (800 IU vitamin E, 1000 mg vitamin C, 25 mg β-carotene)</td>
<td>Yes (10 mg simvastatin, 250–1000 mg niacin)</td>
<td>Yes</td>
<td>Yes</td>
<td>3.2</td>
<td>CVD incidence</td>
<td>5</td>
</tr>
<tr>
<td>You, 2001 (31) and Gaul, 1998 (54)</td>
<td>China</td>
<td>Residents in Linqu</td>
<td>51</td>
<td>47</td>
<td>NR (75)</td>
<td>Yes (200 IU vitamin E, 500 mg vitamin C, 15 mg β-carotene)</td>
<td>Yes (800 mg garlic extract, 4 mg garlic oil)</td>
<td>Yes</td>
<td>Yes</td>
<td>3.3</td>
<td>CVD mortality</td>
<td>5</td>
</tr>
<tr>
<td>Hercberg, 2004 (32)</td>
<td>France</td>
<td>Healthy adults</td>
<td>39</td>
<td>48</td>
<td>Selenium yeast (100)</td>
<td>Yes (30 mg vitamin E, 120 mg vitamin C, 6 mg β-carotene, 20 mg Zn)</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>7.5</td>
<td>CHD incidence</td>
<td>5</td>
</tr>
<tr>
<td>Stranges, 2006 (33)</td>
<td>USA</td>
<td>Patients with skin carcinoma and CVD-free</td>
<td>71</td>
<td>62</td>
<td>Selenium yeast (200)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>7.6</td>
<td>CHD incidence</td>
<td>5</td>
</tr>
</tbody>
</table>

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1 AMI, acute myocardial infarction; NR, not reported; CVD, cardiovascular disease.

2 Quality score based on criteria by Jadad et al (37). Score ranges from 0 (lowest quality) to 5 (highest quality).
for randomized evidence. However, the small number of selenium trials and their relatively small sample size resulted in wide CIs; therefore, beneficial or harmful cardiovascular effects could not be ruled out. In addition, selenium was often used in combination with other vitamins or minerals, which makes it impossible to isolate the specific effects of selenium or of different selenium forms in those trials.

Several trials of selenium supplementation conducted in Chinese populations with low intakes of a variety of vitamins and minerals, including selenium, could not be included in this meta-analysis. Three of these trials reported only cancer outcomes (72–74). Two other trials conducted in Linxian, China, reported cerebrovascular disease but not coronary heart disease or total cardiovascular disease. In these trials, the relative risks of cerebrovascular disease mortality in a comparison of participants receiving 50 μg selenium/d in combination with vitamin E and β-carotene with participants receiving placebo were 0.90 (95% CI: 0.76, 1.07) in healthy participants (75) and 0.62 (0.37, 1.06) in participants with esophageal dysplasia at baseline (76). The relevance of these findings to the effects of selenium in coronary heart disease prevention in Western populations is uncertain. Finally, a randomized trial conducted in institutionalized elderly patients in France evaluated the efficacy of 100 μg selenium/d in combination with zinc in improving immune function and lowering the rate of infections (77). Although coronary heart disease endpoints were not available, the relative risk of total mortality after a 2-y follow-up in participants receiving selenium supplements compared with those receiving placebo was 1.14 (95% CI: 0.91, 1.37).

In conclusion, observational studies showed an inverse association between selenium concentrations and coronary heart disease incidence, but the validity of this evidence is uncertain. Randomized trials, on the other hand, are still inconclusive with respect to the effect of selenium supplementation. The ongoing Selenium and Vitamin E Cancer Prevention Trial, a placebo controlled trial that is testing the effects of 200 μg selenium/d in 32 400 men in the United States and Canada (78), will provide more definitive evidence. The results of this trial are scheduled to appear in 2013. Until then, the observational evidence that low selenium concentrations are a cardiovascular risk factor should be treated as suggestive but not definitive. Furthermore, the public should be warned against the use of selenium supplements for cardiovascular disease prevention. The benefits of selenium supplementation are uncertain, and their indiscriminate use carries a risk of toxicity.

EG, AN-A, and GF-M conceived the idea for the study and developed the search strategy. GF-M and AN-A abstracted the data and conducted data analyses. RP-B conducted statistical analyses for and graphical display of the dose-response meta-analysis. All authors contributed to data and analyses verification and to the writing and revision of the manuscript. The authors have no conflict of interest to declare.

REFERENCES

8. Longnecker MP, Stram DO, Taylor PR, et al. Use of selenium concentra-
tion in whole blood, serum, toenails, or urine as a surrogate measure of
indicator of selenium intake among middle-aged men in an area with low
mentation with different combinations of an association of antioxidant
nutrients on biochemical parameters and markers of the antioxidant
defense system in the elderly. The Geriatrie/Min.Vit.Aox Network. Eur
11. Luoma PV, Sotaniemi EA, Korpela H, Kumpulainen J. Serum selenium,
glutathione peroxidase activity and high-density lipoprotein cholesterol-
mentation on platelet function: a randomized pair-matched, placebo-
controlled, double-blind trial in men with low antioxidant status. Am J
in oxidation resistance of atherogenic serum lipoproteins following an-
tioxidant supplementation: a randomized double-blind placebo-
vitamins, or the combination for the prevention of coronary disease.
15. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Associa-
tion between cardiovascular death and myocardial infarction and serum
selenium concentrations in a 25-year follow-up of Swedish 50-year-old
tration related to myocardial infarction and fatty acid content of serum
Serum selenium and the risk of coronary heart disease and stroke. Am J
Epidemiol 1985;122:276–82.
18. Salonen JT, Salonen R, Penttila I, et al. Serum fatty acids, apolipo-
proteins, selenium and vitamin antioxidants and the risk of death from
19. Ringstad J, Thelle D. Risk of myocardial infarction in relation to serum
concentrations of selenium. Acta Pharmacol Toxicol (Copenh) 1986;
59(suppl):336–9.
20. Kok FJ, de Bruin AM, Hofman A, Valkenburg HA. Selenium status and
chronic disease mortality: Dutch epidemiological findings. Int J Epide-
21. Ringstad J, Jacobsen BK, Thomassen Y, Thelle DS. The Tromso Heart
Study: serum selenium and risk of myocardial infarction a nested case-
22. Suadicani P, Hein HO, Gystelberg F. Serum selenium concentration and
risk of ischaemic heart disease in a prospective longitudinal study. Lancet
23. Miettinen TA, Alfthan G, Huttunen JK, et al. Serum selenium concen-
tration related to myocardial infarction and fatty acid content of serum
vitamins, or the combination for the prevention of coronary disease.
25. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Associa-
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mentation with different combinations of an association of antioxidant
nutrients on biochemical parameters and markers of the antioxidant
defense system in the elderly. The Geriatrie/Min.Vit.Aox Network. Eur
27. Wei WQ, Abnet CC, Qiao YL, et al. Prospective study of selenium concentra-
tions and esophageal and gastric cardia cancer, heart disease,
28. Akbaraly NT, Arnaud J, Hjingier-Favier I, Gouret V, Roussel AM, Ferr
C. Selenium and mortality in the elderly: results from the EVA study.
29. Korpela H, Kumpulainen J, Jussila E, et al. Effect of selenium supple-
mentation after acute myocardial infarction. Res Commun Chem Pathol
30. Kuklinski B, Weissenbacher E, Fahrnrich A. Coenzyme Q10 and anti-
oxidants in acute myocardial infarction. Mol Aspects Med 1994;15(sup-
the progression of precancerous gastric lesions: compliance, serum micro-
nutrients and S-allyl cysteine levels, and toxicity. Eur J Cancer Prev
32. Hńberg S, Galan P, Preziosi P, et al. The SU.VI.MAX Study: a ran-
donized, placebo-controlled trial of the health effects of antioxidant
mentation on cardiovascular disease incidence and mortality: secondary
35. Rajpathak S, Rimm E, Morris JS, Hu F. Toenail selenium and cardio-
36. Longnecker MP, Berlin JA, Orza MJ, Chalmers TC. A meta-analysis of
alcohol consumption in relation to risk of breast cancer. JAMA 1988;
of randomized clinical trials: is blinding necessary? Control Clin Trials
1996;17:1–12.
42.
39. Akesson B, Steen B. Plasma selenium and glutathione peroxidase in
relation to cancer, angina pectoris and short-term mortality in 68-year-
Serum selenium deficiency in myocardial infarction and congestive car-
41. Salonen JT, Salonen R, Seppanen K, et al. Relationship of serum sele-
nium and antioxidants to plasma lipoproteins, platelet aggregation and
persistent ischaemic heart disease in Eastern Finnish men. Atheroscle-
43. Thiele R, Schuffenhauer M, Winnefeld K, Dawcynski H, Pleissner J,
Pfifer R. [Selenium level in patients with acute myocardial infarction and
in patients with severe angina pectoris without myocardial infarction]. Med
Klin (Munich) 1995;90(suppl):45–8 (in German).
44. Coudray C, Roussel AM, Mainard F, Aurnaud J, Favier A. Lipid peroxi-
dation level and antioxidant micronutrient status in a pre-aging popula-
tion; correlation with chronic disease prevalence in a French epidemi-
45. Navarro-Alarcon M, Lopez-Garcia de la Serrana, Perez-Valero V,
Lopez-Martinez C. Serum and urine selenium concentrations in patients
with cardiovascular diseases and relationship to other nutritional in-
46. Bor MV, Cevik C, Uslu I, General F, Duzgun E. Selenium levels and glutathione peroxidase activities in patients with acute myocardial in-
47. Zacharia BA, Ukleja-Adamowicz M, Nartowicz E, Lecka J. Increased
cardiac and coronary microvascular alcoholic myocardial infarction, an
48. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-
49. Greenland S, Longnecker MP. Methods for trend estimation from sum-
morized dose-response data, with applications to meta-analysis. Am J
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morized dose-response data, with applications to meta-analysis. Am J


### APPENDIX A

Quality criteria for evaluating the design and data analysis of observational studies on selenium and coronary heart disease

<table>
<thead>
<tr>
<th>All observational studies</th>
<th>Prospective cohort studies (reference number)</th>
<th>Case-control studies (reference number)</th>
</tr>
</thead>
</table>
| Exposure was assessed at the individual level | ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■...