Selenium Status and Cardiovascular Diseases: Meta-Analysis of Prospective Observational studies and Randomized Controlled Trials

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Short title: Selenium levels and CVD

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Abstract:

Background Selenium was thought to play a role in cardiovascular disease (CVD) due to its antioxidant properties; however, evidence from observational studies and randomized controlled trials (RCT) has been inconsistent and controversial. We thus conducted a meta-analysis to assess the discrepancies between observational and randomized trial evidence.

Method We searched MEDLINE and EMBASE for eligible prospective studies regarding the relationship between selenium and CVD up to December 15, 2013 and finally included 16 prospective observational studies and 16 RCTs. Random effects model was used to estimate the pooled relative risk (RR). Generalized least-squares trend test and restricted cubic spline model was performed to assess a linear and non-linear dose-response relation.

Results Our meta-analysis of prospective studies showed a non-linear relation of CVD risk with blood selenium concentrations across a range of 30-165μg/L and a significant benefit of CVD within a narrow selenium range of 55-145μg/L. Our meta-analyses of RCTs showed that oral selenium supplements (median dose: 200μg/day) for 2 weeks to 144 months significantly raised blood selenium concentrations by 56.4μg/L (95% CI: 40.9, 72.0μg/L), whereas oral selenium supplements (median: 100μg/day) for 6 to 114 months caused no effect on CVD (RR=0.91; 95% CI: 0.74, 1.10).

Conclusion Our meta-analysis in prospective studies demonstrated a non-linear inverse association between selenium status and CVD risk within a narrow selenium range, whose upper bound was over-elevated by raised selenium after supplementation and a null effect was observed in RCTs. These findings indicate the importance of considering selenium status, dose and safety in future trials.

Keywords: prospective observational studies; randomized controlled trials; selenium; meta-analysis; cardiovascular disease.

Introduction

Selenium exerts its biological functions on redox signaling, antioxidant defense, immune response, and thyroid hormone function mainly via selenium-dependent glutathione peroxidases (GPx) and other selenoproteins [1-4]. Adequate intake of selenium may be beneficial for cardiovascular disease (CVDs), cancer, and other chronic diseases [5, 6]. Food is the primary source of selenium contents in the human body; however, dietary selenium intake varies widely and primarily depends on the soil on which crops and fodder are grown [7]. Selenium was added to various dietary supplements as a popular supplement [5], although the prevention effects on CVD have not been confirmed.

There is a longstanding interest in the CVD research community regarding the potential yet unproven benefits or risks of selenium intake on the development and progression of CVD. There were largely divergent results between the observational studies and RCTs. Earlier retrospective case-control studies showed that blood selenium concentrations of CVD patients were lower than those of healthy population, indicating an inverse correlation [8, 9]. A significant inverse association between selenium status and risk of coronary heart disease was reported in a meta-analysis of 25 observational studies [8], yet there has been little research on whether there is a threshold effect for the relation between selenium concentrations and CVD events. Individual observational studies have shown inconsistent findings and have not fully considered the possible nonlinear relationship. Also, influenced by other antioxidants cannot be ruled out in observational studies. Well designed and conducted RCTs, as the most reliable design strategy, can avoid most of biases inherent in observational studies and help evaluate a possible causal relation. However, a few randomized trials have evaluated the effects of

selenium on cardiovascular outcomes [10-13] and showed no obvious benefits from selenium for CVD. In addition to heterogeneity in intervention periods and selenium formula and dosage, these individual trials are limited by statistical power for addressing specific thresholds of circulating selenium concentrations for optimal cardiovascular health. Previously, neither of a meta-analysis of 6 RCTs [8] for selenium-containing supplements and a meta-analysis of 12 RCTs for selenium supplements alone [14] showed significant protective effect on cardiovascular endpoints. Both the meta-analyses focused on testing the selenium-CVD hypothesis but did not specifically address the dose-dependent relation. There is still disagreement between observational studies and RCTs, which largely hindered a consistent conclusion to be drawn.

To maximize statistical power and reduce sampling bias from individual studies, we conducted a meta-analysis of available prospective data from both observational studies and RCTs. Specifically, our study aimed to provide a comprehensive evaluation of the full spectrum of variation in baseline selenium concentrations and its dose-response relationship with incident CVDs in prospective observational studies, and determine whether any differences in selenium biomarkers by selenium supplementation could account for CVD risk in RCTs.

Methods

Data source and searches

We searched MEDLINE and EMBASE databases for all relevant articles on selenium and cardiovascular disease published up to December 15, 2013. We used the search terms

including “selenium”, “selenite”, “selenate”, “cardiovascular disease”, “myocardial infarction”, “stroke”, “peripheral arterial disease”, “mortality”, “coronary heart disease”, “ischemic heart disease”, “sudden cardiac arrest”, “cardiovascular risk”, “hypertension”, “cholesterol”, “hypercholesterolemia”, “hyperlipidemia”, “diabetes”, “arteriosclerosis” and “hypertriglyceride”. The search was restricted to English-language only and adults.

We chose the articles based on the following inclusion criteria: 1) original studies (not reviews, meeting abstracts, editorials, letters or commentaries); 2) adult human studies; 3) prospective study design (eg, prospective cohort, nested case-control, case-cohort) or RCTs; 4) prospective studies that provided the relative risk estimation between baseline circulating or toenail selenium concentration and CVD incidence or mortality; and 5) RCTs with selenium-containing supplements (selenium alone or a combination with other vitamins or minerals), which provided available data of selenium dose and CVD incidence or mortality and/or circulating concentrations of selenium or selenium protein GPx activity. We also manually searched bibliographies from recent reviews and retrieved articles for additional studies. Finally, a total of 16 articles of prospective observational studies and 16 articles of RCTs were included in this meta-analysis.

Data extraction

Two investigators (X Zhang and C Liu) independently selected articles and extracted the data. Any discrepancies were resolved by consensus. Information extracted from articles included population source, study design, follow-up period, sample size, subject characteristics (age and sex), selenium biomarkers, CVDs endpoints, selenium forms and dose (RCTs). When results were available on different subpopulations in the same cohort [1, 3,

[10, 15-17] and single RCT [18, 19], we considered each subpopulation as an independent study in the meta-analysis (basic study characteristics were described in **supplemental table 1** for prospective studies and in **supplemental table 2** for RCTs).

Of 16 prospective observational studies, most of them (14) provided RRs or hazard ratios and 95% CIs for the relation between baseline selenium concentrations and CVDs events. Two articles provided RRs for selenium concentrations as a continuous variable were not included in the analysis due to uncertain comparison scales [20, 21]. We alternatively calculated crude RRs in the studies that only provided exact numbers of events [16, 17] and chose RRs estimated from the models fully adjusted for major confounders as main results in the articles with several estimation models.

Statistical analysis

We analyzed observational studies and RCTs respectively and estimated the pooled RRs by DerSimonian and Laird's random effect model in which each study was weighted by the inverse of sum of within-study plus between-study variance [22]. Between-study heterogeneity was tested by Cochrane's Q statistic, I^2 and H statistics, respectively. The percentages of I^2 around 25% (I^2=25), 50% (I^2=50), and 75% (I^2=75) indicate low, medium, and high heterogeneity, respectively. An H statistics <1.2 indicates little heterogeneity and an H >1.5 raises caution regarding notable heterogeneity. We used Begg's adjusted rank correlation test and Egger's regression asymmetry test to test publication bias [23, 24].

For observational studies, we also explored differences of the pooled RRs from baseline measurements, including sex (women, men, or mixed), age (<60y and ≥60y), sample size

(<1000 and ≥1000), covariance adjustment (BMI and smoking), and CVD endpoints (CVD, CHD, MI, and stroke).

We used the method proposed by Greenland and Longnecker [25] to assess the linear relationship of selenium concentrations and CVD risk. To explore a possible non-linear trend, we first graphically examined the relation shape by using LOWESS smoothed curve and quadratic curve; second, we applied the 2-stage random-effect dose-response meta-analysis method proposed by Orsini with 3 fixed knots at percentiles of 10^{th}, 50^{th}, and 90^{th} for the distributions of reported circulating selenium concentrations across all included studies [26, 27]. The concentration values of each category were determined as the median or mean concentrations if available; otherwise we calculated the means or midpoints of the lower and upper bounds instead. If there was an open lower or upper-bound, it was estimated by one known bound minus or plus the other half width of the adjacent category.

For RCTs, the pooled RRs for the overall effect of selenium supplementation on CVD events were calculated. We then examined whether sample size (<1000 and ≥1000), trials duration (≤5y and >5y), selenium supplements (selenium alone and a combination of selenium with other antioxidants), supplemental dose (≤100μg/day and 200μg/day), and selenium formulation (bio-selenium and all others) modified the association. Changes in blood selenium concentrations in response to supplementation were derived, respectively, in 6 trials with ≤100μg/day supplements and 4 trials with 200-300μg/day supplements. We calculated the weight mean difference of circulating selenium concentrations comparing the treatment to the placebo groups.

All analyses were performed using the STATA software (version 13, STATA Corp., College Station, Texas). Statistical significance was defined as two-tailed $\alpha<0.05$.

Results

A total of 16 prospective studies involving 35 607 participants and 4 421 incident CVD cases were included in this meta-analysis (**Figure 1**). Of them, 11 were cohort studies and 6 were nested case-control studies. Most studies (13 studies) were population-based and 3 were health professional populations [13, 28, 29]. Biospecimen tissues for selenium concentrations included serum (13 studies), erythrocyte (1 study) [30], plasma (1 study) [29], and toenail (2 studies) [13, 28].

Of all 16 trials, 37 572 participants (range: 23 to 17 448; median: 351) took the median dose of 100μg/day (range: 75 to 300μg/day) selenium supplements for 2 weeks to 114 months duration (median: 12 months). 14 of all trials were placebo-controlled double-blinded design and 2 used open label design [31, 32]. Selenium formulation included L-selenomethionine [33-35], sodium selenite [36, 37] and selenium-enriched yeast [33, 38]. One study did not report form information [39]. Of all included trials, 9 trials estimated RRs of CVDs mortality or incidence, **(Supplemental table 2)**, 10 trials reported information of selenium biomarkers, and only 3 trials [33, 39, 40] provided both.

Selenium Concentrations and CVD Events in Prospective Observational Studies

By combining evidence from 16 studies, the pooled RR for the highest (median: 101.5μg/L) versus the lowest category (median: 53.7μg/L) of baseline blood (serum/plasma/erythrocyte) selenium concentrations was 0.87 (95% CI: 0.76, 0.99),

indicating a significant but modest association between baseline selenium concentrations and CVD risk (**Figure 2**). Neither publication bias nor between-study heterogeneity was statistically significant. In stratified analyses (**Table 1**), none of sex, follow-up duration, sample size, specimen type, adjustment for BMI or smoking, and baseline selenium concentrations seemed to materially modify the inverse association. The inverse associations were more evident among those studies with lower median or mean baseline selenium concentrations (<106μg/L) (RR, 0.77; 95% CI: 0.61, 0.96) than those with higher (≥106μg/L) (RR, 0.93; 95% CI: 0.80, 1.10), but the interaction was not significant (P=0.14). In addition, there was no evidence for significant relation between toenail selenium concentrations and CVD based on 2 studies (**Figure 2**).

The overall dose-response relation was assessed across the range of selenium concentrations between 30μg/L and 165μg/L. For each 25μg/L increment in circulating selenium concentrations, the pooled RR was estimated to be 0.89 (95% CI: 0.84, 0.95). The analysis modeled by restricted cubic spline suggested a reasonably nonlinear relationship between circulating selenium and CVD risk (**Supplemental Figure 1**). The curve showed that selenium concentrations were significantly associated with lower risk of CVD at a range from 55 to 145μg/L with a nadir at 125μg/L as compared with low selenium concentrations (median: 53.7μg/L) (**Figure 3**). The association was the null when it exceeded 145μg/L. Evidence was insufficient to examine the relation between selenium concentration and CVD risk when selenium concentration exceeded 150μg/L.

Selenium Supplementation and CVD Events in RCTs

Our meta-analysis of 9 RCTs showed that oral selenium supplements (75- 300μg/day, median: 100μg/day) for 6 to 114 months (median: 60 months) did not significantly decrease the incidence of CVD events (RR=0.91; 95% CI: 0.74, 1.10) as compared with the placebo groups (**Table 2**). There was a weakly significant between-study heterogeneity (P for Cochran Q test=0.07, H statistics=1.4 (1.0, 2.0), and I^2 =45 (0, 75)). The Begg's funnel plot showed that the smaller RRs with small standard errors tended to be near the null effect line, and larger RRs with large standard errors tended to be under the horizontal line. This indicated the presence of publication bias in favor of small trials with positive findings (Egger test, P=0.03; Begg's test P=0.10). In the stratified analyses (**Table 2)**, smaller trials with shorter trial durations tended to report positive results; the pooled RR was 0.42 (95% CI, 0.24, 0.73) for small trials (<1000) with duration ≤ 5 years, and 1.02 (95% CI, 0.93, 1.11) for large trials (≥1000) with duration >5 years (P for interaction=0.002). In addition, differences in mean ages of participants, study area, selenium formulation, supplemental doses, and CVD endpoints did not appear to change the risk of CVD by selenium supplementation. The pooled RR was 0.78 (95% CI: 0.49, 1.26) for 6 trials with dose of ≤100μg/day (only one is 75μg/day [41, 42]) and 0.91 (95% CI: 0.69, 1.21) for 3 trials with 200μg/day selenium intake (**Figure 4**).

Selenium Biomarker Concentrations in Response to Selenium Supplementation

Our meta-analysis of 10 RCTs showed that oral selenium supplements (median dose: 200μg/day) for 6.5 months (range: 2 weeks to 144 months) significantly raised blood selenium concentrations by 56.4μg/L from a median baseline selenium concentrations of 98.5μg/L (95% CI for weighted mean differences [WMD]: 40.9, 72.0μg/L). Different

formulations of selenium supplements had non-significant effects on the circulating selenium concentrations, thus we pooled all trials with different formulations of supplements to address following does-response relationship. A steep linear relationship between supplemental duration and concentration changes for dose of 100μg/day before 9 month supplementation (**Supplemental Figure 2**). A similar relationship was showed for dose of 200μg/day before 13 months after supplementation and then a plateau change between 90 and 110μg/L was reached. However, there are not enough data to address the plateau for the dose of 100μg/day.

Since the cardiovascular health by selenium is thought to be through antioxidant function of GPx, we have additionally examined available data from 5 RCTs to character a time course of percentage changes of GPx activity in blood after selenium supplementation. Percentage changes of GPx activity increased abruptly at 1-2 weeks after oral selenium supplement and then reached the maximal levels at 12 weeks (**Supplemental Figure 3**).

Discussion

Our meta-analysis of prospective observational studies provided some evidence of a possible non-linear, most likely U-shaped, relationship between baseline selenium concentrations and CVD. Within a narrow range from 55 to 145μg/L, selenium concentrations were associated with a significantly lower risk of CVD. We found no evidence for significant effect modifications by sex, follow-up duration, sample size, specimen type, baseline selenium concentrations, and adjustment for BMI or smoking. Our meta-analysis of RCTs showed no evidence for an overall effect of oral selenium supplements on CVD events with a 44% elevation of selenium concentrations. Neither selenium formulation nor dose

(100μg/day or 200μg/day) modified this effect. In addition, evidence for publication bias indicated that smaller RCTs with positive results may largely account for this significant effect on CVD by selenium supplementation as previously reported.

A previous meta-analysis of prospective observational studies reported a similar inverse association between CHD and selenium concentrations although the influence of other antioxidants cannot be ruled out in observational studies [8]. Selenium status may possibly affect this relationship [8] and this relation might be discernible only in a population with lower selenium concentrations. The narrow selenium range of CVD reduction (55 to 145μg/L) reported by our meta-analysis was similar to the range of adequate selenium levels at 60-140μg/L as previously reported [6]. Due to limited data, we only addressed the non-linear relation when selenium concentration did not exceed 150μg/L. Further studies for exact boundary of this relationship are warranted.

The non-linear associations might be influenced by many potential factors, such as sample size, duration, specimen types, and baseline selenium status. Adjustment for BMI and smoking did not change the strength of the associations, although they were potential confounders [43, 44]. The median level of blood selenium from observational studies included in our meta-analysis was 102.8μg/L, which was slightly lower than that in a nationally representative sample of the US population from the NHANES 2003-2004 (136.4 ± 19.9μg/L) [45]. The source of biospecimen for assessment may modify this association [45-47]. However, there was a small number of studies that assayed biospecimen samples other than serum. Also, we were unable to exclude the non-linear association that might be caused by statistical fluctuation due to relatively low power. In addition, several lines of evidence seem

to support the hypothesis of non-linear relationship between selenium and CVD. For instance, a randomized controlled pilot trial of 501 old persons with low selenium status found that low dose of selenium supplementation had a significant effect on decreasing total and non-HDL cholesterol concentrations, while the effect was non-significant for a high dose supplementation (300μg/day) [3]. Similarly, a 57% higher risk of diabetes was observed in the highest quintile of serum selenium (147.0μg/L) compared with the lowest quintile (105.9μg/L) in the NHANES III [49]. Taken together, it seems reasonable to speculate that high selenium concentrations may be related to elevating levels of some intermediate CVD risk factors, including dyslipidemia and type 2 diabetes, and may thus diminish the inverse association and even lead to possibly increased risk of CVD risk. Nevertheless, few prospective studies have specifically assessed this hypothesis.

Our meta-analysis of RCTs found that oral selenium supplements had no significant effect on CVD, which was consistent with previous meta-analyses [8, 14]. Publication bias in previous RCTs may possibly explain the observed significant results in some individual trials. In particular, most large trials with longer durations reported null findings suggested that substantial publication bias due to selective publication of small trials with positive results is likely.

The null effect of selenium supplementation on CVD risk was also complicated by the significant between-study heterogeneity in selenium dosage, formula, duration, and combinations of supplements. Selenium dosage varied across individual RCTs. These differences might have contributed to differential results and led to difficulties in estimating the true effect of optimal dose of selenium supplements. Our results clearly show that oral

selenium supplements, either dose of 100μg/day or 200μg/day, significantly increases selenium concentrations and thereby can replete selenium status in human body. It should be noted that circulating selenium after 12 weeks of selenium supplementation were significantly elevated by at least 50μg/L comparing with placebo and raised by 150μg/L above a median baseline concentrations of 100μg/L. The median of circulating selenium concentrations was 123.6μg/L with an interquartile range from 113.7 to 134.7μg/L in a nationally representative sample of the US general populations aged ≥20 years, a US. National survey data of NHANES III (1988-1994) with 7129 participants [50]. In the present study, the median circulating levels of all 10 included trials were 97μg/L (interquartile range: 90-108μg/L) at baseline, which were slightly lower than the levels of NHANES III. After oral selenium supplementation (with a median dose of 200 mg/day for a median duration of 6 months), circulating selenium concentrations increased to a median level of 150μg/L (interquartile range: 135- 225.7μg/L), which were apparently higher than the estimated levels and ranges from NHANES III data. Based on above available evidence, it seems reasonable to conclude that significantly elevated selenium concentrations by taking selenium supplements at a dose ≥100μg/day were above the range of 55-145μg/L associated with significant risk reduction and may not be optimal for CVD health. However, no statistical significant of CVD risk was found, although response levels of selenium were significant higher, for higher dose of 200mg/day vs. lower dose of 100mg/day. Nevertheless, evidence from a dose ≥300μg/day has been limited and inconclusive. Only one RCT reported a similarly significant increment of selenium concentrations by 41.2μg/L (29.9-51.3) after a higher dose of 300μg/day selenium supplementation for 12 weeks [37]. The trial duration might

be another potential source of heterogeneity. We observed a significant difference between subgroups of duration ≤ 5y and > 5y and a time-dependent change of serum selenium in response to supplementation, although such a difference might be caused by chance due to small sample sizes in subgroups.

In addition, available evidence indicates that the role of selenium in human health is primarily due to its presence in selenoproteins, including antioxidant enzyme glutathione perosidase (GPx), although the exact mechanisms have not yet been fully elucidated. The hypothesis of selenium and CVD is supported by the ability of GPx to combat the oxidative modification of lipids and to reduce platelet aggregation [5]. The findings from our meta-analysis of GPx activity may explain disparate results between observational studies and RCTs for cardiovascular health by selenium. Our meta-analysis of GPx activity showed that 12-week selenium supplementation caused a maximal increment in GPx activity by 12%. However, it remains uncertain whether increment is sustained in the long-term period and contributes to the effects of selenium on CVD, due to limited numbers of RCTs with available data on GPx activity.

There is also a concern on the effect of selenium forms of supplements on circulating selenium concentrations. Evidence supported that the bio-available of organic selenium is superior to that of inorganic selenium because inorganic selenium may increase the oxidant stresses [51]. Due to limited power, it is difficult to tease out the effect of selenium forms in our study. Besides, differences in study population, intervention periods, CVD events, and selenium status might have decreased overall statistical power for testing the hypothesis whether selenium intake from various supplements exerts any beneficial effect on CVD

events.

Our meta-analysis has several limitations. First, the observational nature of prospective studies included in our analysis cannot rule out residual confounding, although the consistency of our results across multiple strata and sensitivity analyses minimizes the likelihood that residual confounding explains the findings. Second, all included observational studies used a single measurement of selenium at baseline, which is not a time-integrated measure of selenium status and thereby affect the association. Third, substantial between-study heterogeneity could influence the accuracy in the pooled estimates. Nevertheless, the strength and the direction of the associations were essentially unchanged after excluding the studies with extreme values. Fourth, as in any meta-analysis, publication bias is possible, although we attempted to retrieve all relevant data. Fifth, the benefits of selenium may only present in the deficient population. Due to sparse data, we have insufficient statistical power to clearly illustrate this hypothesis. Also we have low power to explore the differential effects between selenium supplements alone and combined selenium supplements. Finally, limited data from existing prospective studies and RCTs provided insufficient power to detect potential sources of heterogeneity and interactions. Additionally, we cannot completely exclude the possibility that changes in treatment compliance for all the trials included and differential serum selenium concentrations in response to supplementation which may affect the explanation for our observed differences between treatment and placebo, especially when relevant information was unavailable and trial duration was long.

Conclusions

Our meta-analysis of 16 prospective observational studies suggested a non-linear relation between baseline blood selenium concentrations and risk of incident CVDs, the significant benefit range of selenium concentration was limited from 55 to 145μg/L. Our meta-analysis of 9 RCTs found no overall effect of oral selenium supplements on CVD with significantly elevated selenium concentrations at a mean level of approximately 154μg/L, which was above the upper limit of the observed beneficial range (145μg/L). Our findings thus indicated a need of future long-term RCTs with optimal selenium supplemental dose and safety considerations. At presence, available evidence is not conclusive to support the widespread use of selenium or selenium-containing supplements for CVD prevention.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Information

Supplementary information is available at EJCN's website.

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Figure legends

Figure 1. Flow chart of study selection

Figure 2. A random-effect meta-analysis of 16 independent prospective studies with adjusted relative risk (*RR*) and 95% confidence interval (CI) of CVDs in relation to blood or toenail selenium concentrations (the highest versus the lowest category).

Figure 3. Dose-response relation between baseline concentrations of selenium and the risk of CVDs in 16 independent prospective studies. The relation is fitted by the quadratic regression model. Circles indicate *RR* in each study. The circle size is proportional to the precision of the *RR* (inverse of variance). The grey shaded region shows the 95% CIs around the regression line. The selenium concentrations were across the range from 30.5 to 164.6μg/L; the median concentrations in all the control groups were 53.7μg/L.

Figure 4. A random-effect meta-analysis of 9 independent RCTs with adjusted relative risk (*RR*) and 95% CI of CVDs in relation to selenium supplementation (active selenium treatment group versus placebo group).

* Selenium supplemental dose of W C You (2001) was 75μg/day.

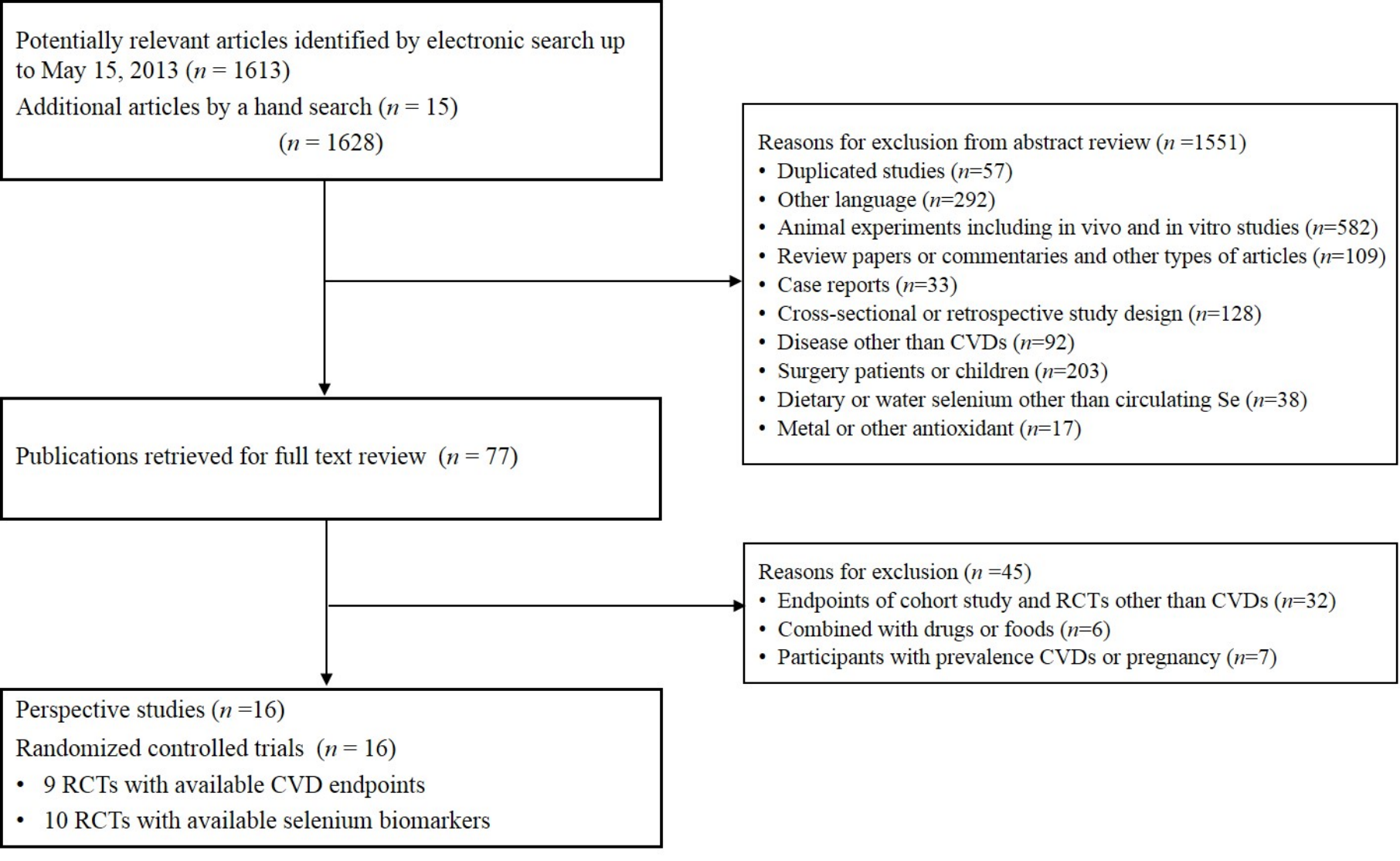
Table 1. Meta-analysis of Prospective Observational Studies that Examined the Association between Blood (Serum/Plasma/erythrocyte) Selenium Concentrations and CVD Events

	No. of studies	Summary of RR 95% CI	P for heterogeneity			P for Interaction
			Q test	H	I^2	
All studies	14	0.87 (0.76, 0.99)	0.47	1.0 (1.0, 1.5)	5 (0, 57)	
Sex						0.26
Men	5	0.86 (0.73, 1.01)	0.10	1.4 (1.0, 2.3)	49 (0, 81)	
Men and Women	9	0.95 (0.89, 1.02)	0.39	1.0 (1.0, 1.7)	6 (0, 67)	
Duration of follow-up						0.15
< 10 y	8	0.75 (0.58, 0.97)	0.34	1.1 (1.0,1.9)	11 (0, 71)	
≥10 y	6	0.94 (0.81, 1.09)	0.68	1.0 (1.0, 2.0)	0 (0, 75)	
Sample size						0.22
< 1000	7	0.98 (0.76, 1.28)	0.75	1.0 (1.0, 1.9)	0 (0, 71)	
≥ 1000	7	0.80 (0.65, 0.98)	0.16	1.2 (1.0, 1.9)	35 (0, 73)	
Baseline Selenium Concentrations (μg/L)						0.14
< 106	7	0.77 (0.61, 0.96)	0.42	1.0 (1.0, 1.9)	1 (0, 71)	
≥ 106	7	0.93 (0.80, 1.10)	0.49	1.0 (1.0, 1.9)	0 (0, 71)	
Specimen						0.28
Serum	11	0.83 (0.70, 0.98)	0.31	1.1 (1.0, 1.5)	15 (0, 55)	
Others	3	1.00 (0.60, 1.68)	0.30	1.0 (1.0, 3.1)	0 (0, 90)	
Adjustment for BMI						0.76
No	11	0.85 (0.72, 1.01)	0.51	1.0 (1.0, 1.6)	0 (0, 60)	
Yes	3	0.80 (0.54, 1.17)	0.13	1.4 (1.0, 2.7)	51 (0, 86)	
Adjustment for smoking						0.38
No	9	0.91 (0.74, 1.12)	0.74	1.0 (1.0, 1.7)	0 (0, 65)	
Yes	5	0.78 (0.59, 1.03)	0.08	1.4 (1.0, 2.4)	52 (0, 82)	
CVD Endpoints						0.67
CVD	6	0.88 (0.71, 1.09)	0.39	1.0 (1.0, 2.0)	4 (0, 76)	
CHD	8	0.72 (0.57, 0.92)	0.19	1.2 (1.0, 1.8)	29 (0, 68)	
MI	7	0.81 (0.60, 1.09)	0.75	1.4 (1.0, 2.1)	48 (0, 78)	
Stroke	4	0.69 (0.29, 1.63)	0.003	2.2 (1.3, 3.5)	79 (42, 92)	

Table 2. Meta-Analysis of RCTs that Reported CVD Events for Selenium Supplementation versus Placebo groups

	No. of studies	Summary of RR 95% CI	P for heterogeneity			P for Interaction
			Q test	H	I^2	
All studies	9	0.91 (0.75, 1.11)	0.07	1.4 (1.0, 2.0)	45 (0, 75)	
Supplements						0.21
Selenium	3	1.01 (0.83, 1.22)	0.22	1.2 (1.0, 3.8)	33 (0, 93)	
Combined with other antioxidants	6	0.74 (0.48, 1.15)	0.075	1.4 (1.0, 2.2)	50 (0, 80)	
Geographical Area						0.16
USA	5	1.02 (0.92, 1.12)	0.83	1.0 (1.0, 3.1)	0 (0, 90)	
Europe	3	0.63 (0.33, 1.22)	0.02	1.7 (1.1, 2.8)	67 (14, 87)	
Duration of follow-up						0.004†
≤ 5 y	5	0.49 (0.30, 0.80)	0.36	1.0 (1.0, 2.3)	8 (0, 81)	
> 5 y	4	1.02 (0.93, 1.11)	0.87	1.0 (1.0, 2.6)	0 (0, 85)	
Sample size						0.002†
< 1000	4	0.42 (0.24, 0.73)	0.37	1.0 (1.0, 2.6)	4 (0, 85)	
≥ 1000	5	1.01 (0.93, 1.11)	0.88	1.0 (1.0, 2.2)	0 (0, 79)	
Duration and sample size						0.002†
≤ 5 y and < 1000	4	0.42 (0.24, 0.73)	0.37	1.0 (1.0, 2.6)	4 (0, 85)	
> 5 y and ≥ 1000	4	1.02 (0.93, 1.11)	0.87	1.0 (1.0, 2.6)	0 (0, 85)	
Age						0.53
< 60 y	4	0.93 (0.64, 1.36)	0.28	1.1 (1.0, 2.9)	22 (0, 88)	
≥60 y	5	0.79 (0.55, 1.13)	0.02	1.8 (1.1, 2.8)	67 (15, 87)	
Baseline selenium status						0.19
≤ 100μg/L	3	0.94 (0.50-1.76)	3.52	1.3 (1.0, 2.4)	43 (0, 83)	
> 100μg/L	2	1.02 (0.93-1.13)	0.004	NA	NA	
CVD Events						0.34
MI	3	0.32 (0.07, 1.64)	0.04	1.8 (1.0, 3.3)	68 (0, 91)	
CHD	3	1.00 (0.84, 1.21)	0.71	1.0 (1.0, 3.1)	0 (0, 90)	
CVD	5	0.91 (0.72, 1.14)	0.14	1.3 (1.0, 2.2)	42 (0, 79)	
CVD End Points						0.09
Incidence	4	1.02 (0.93, 1.11)	0.87	1.0 (1.0, 2.6)	0 (0, 85)	
Mortality	7	0.71 (0.47, 1.07)	0.05	1.4 (1.0, 2.2)	52 (0, 79)	

* indicates P<0.05; † indicates P<0.01.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Additional articles by a hand search (n = 15)
(n = 1628)
Reasons for exclusion from abstract review (n =1551)
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• Other language (n=292)
• Animal experiments including in vivo and in vitro studies (n=582)
• Review papers or commentaries and other types of articles (n=109)
• Case reports (n=33)
• Cross-sectional or retrospective study design (n=128)
• Disease other than CVDs (n=92)
• Surgery patients or children (n=203)
• Dietary or water selenium other than circulating Se (n=38)
• Metal or other antioxidant (n=17)
Publications retrieved for full text review (n = 77)
Reasons for exclusion (n =45)
• Endpoints of cohort study and RCTs other than CVDs (n=32)
• Combined with drugs or foods (n=6)
• Participants with prevalence CVDs or pregnancy (n=7)
Perspective studies (n =16)
Randomized controlled trials (n = 16)
• 9 RCTs with available CVD endpoints
• 10 RCTs with available selenium biomarkers

Figure 1.

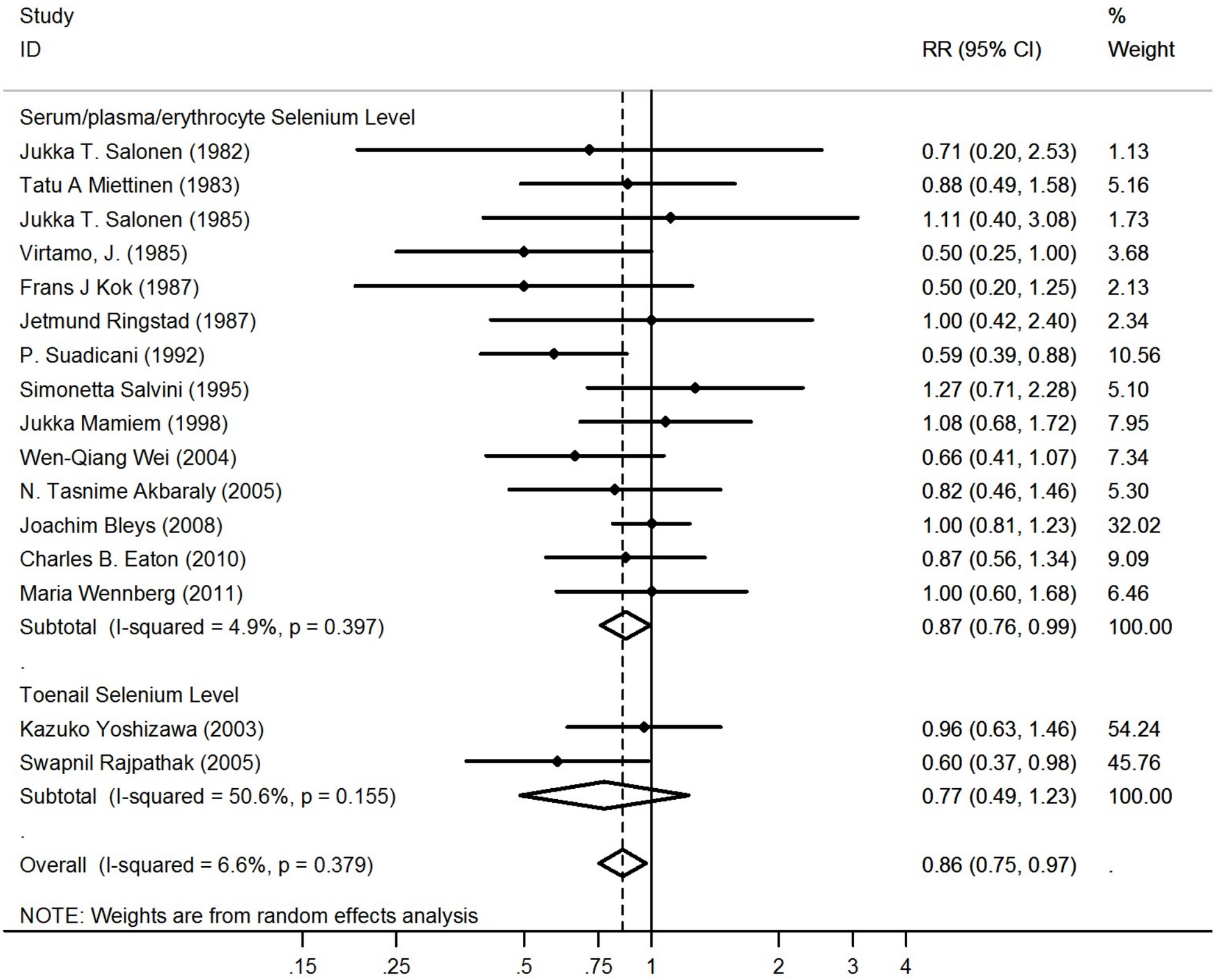

Study
ID
RR (95% CI)
%
Weight
Serum/plasma/erythrocyte Selenium Level
Jukka T. Salonen (1982)
0.71 (0.20, 2.53)
1.13
Tatu A Miettinen (1983)
0.88 (0.49, 1.58)
5.16
Jukka T. Salonen (1985)
1.11 (0.40, 3.08)
1.73
Virtamo, J. (1985)
0.50 (0.25, 1.00)
3.68
Frans J Kok (1987)
0.50 (0.20, 1.25)
2.13
Jetmund Ringstad (1987)
1.00 (0.42, 2.40)
2.34
P. Suadicani (1992)
0.59 (0.39, 0.88)
10.56
Simonetta Salvini (1995)
1.27 (0.71, 2.28)
5.10
Jukka Mamiem (1998)
1.08 (0.68, 1.72)
7.95
Wen-Qiang Wei (2004)
0.66 (0.41, 1.07)
7.34
N. Tasnime Akbaraly (2005)
0.82 (0.46, 1.46)
5.30
Joachim Bleys (2008)
1.00 (0.81, 1.23)
32.02
Charles B. Eaton (2010)
0.87 (0.56, 1.34)
9.09
Maria Wennberg (2011)
1.00 (0.60, 1.68)
6.46
Subtotal (I-squared = 4.9%, p = 0.397)
0.87 (0.76, 0.99)
100.00
.
Toenail Selenium Level
Kazuko Yoshizawa (2003)
0.96 (0.63, 1.46)
54.24
Swapnil Rajpathak (2005)
0.60 (0.37, 0.98)
45.76
Subtotal (I-squared = 50.6%, p = 0.155)
0.77 (0.49, 1.23)
100.00
.
Overall (I-squared = 6.6%, p = 0.379)
0.86 (0.75, 0.97)
.
NOTE: Weights are from random effects analysis
.15
.25
.5
.75
1
2
3
4

Figure 2.

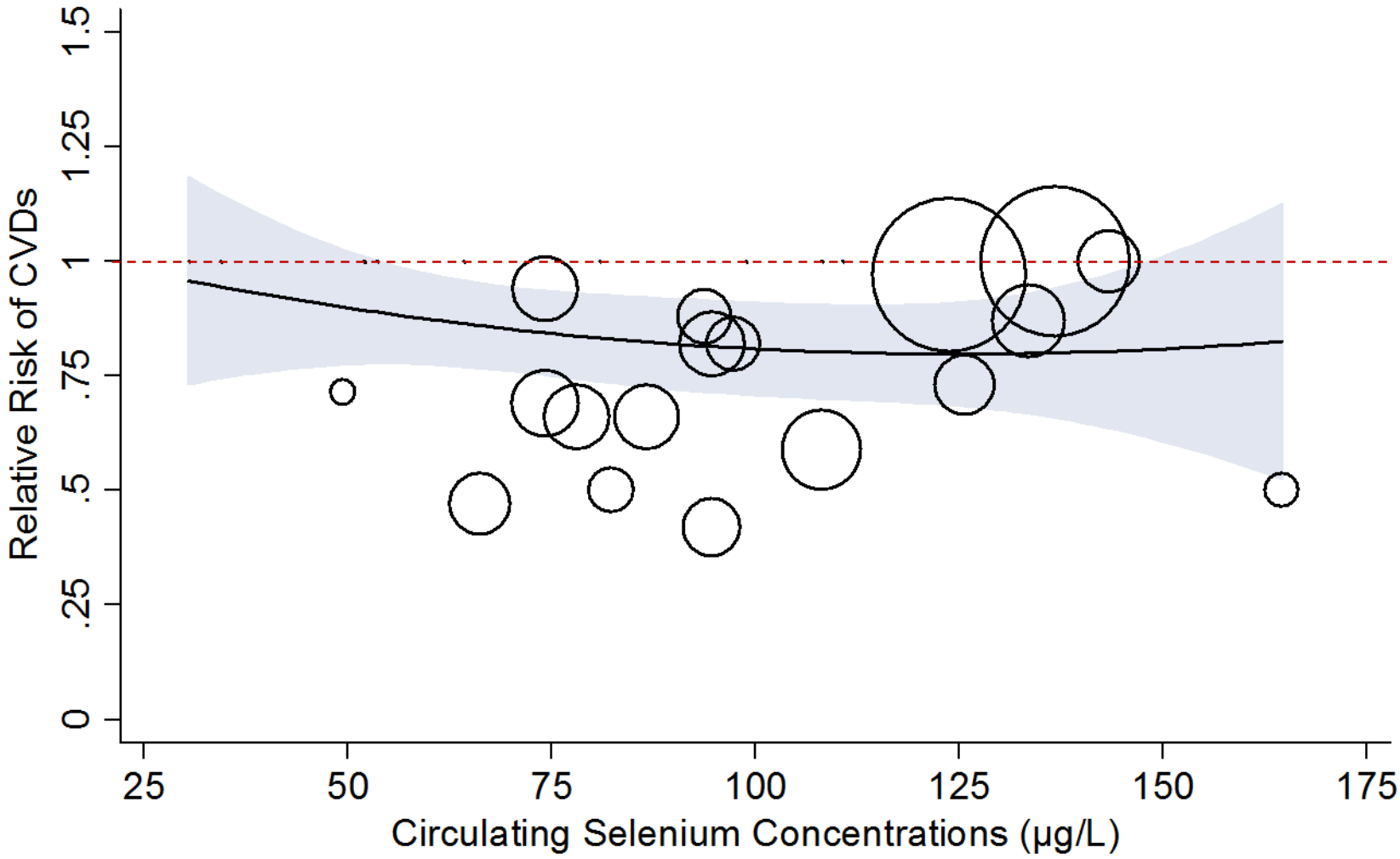

Relative Risk of CVDs
0
.25
.5
.75
1
1.25
1.5
25
50
75
100
125
150
175
Circulating Selenium Concentrations (μg/L)

Figure 3.

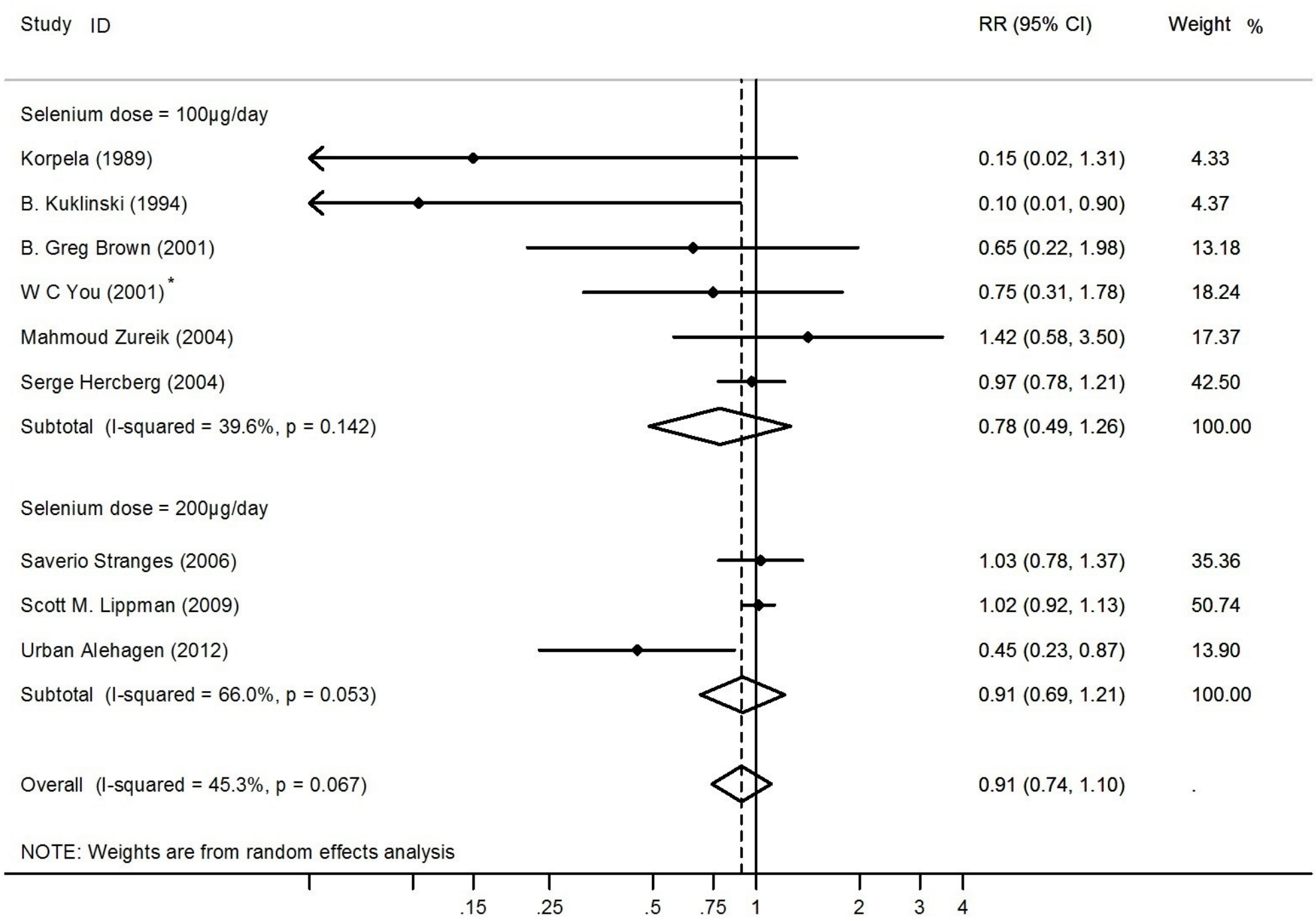

Study ID
RR (95% CI)
Weight %
Selenium dose = 100µg/day
Korpela (1989)
0.15 (0.02, 1.31)
4.33
B. Kuklinski (1994)
0.10 (0.01, 0.90)
4.37
B. Greg Brown (2001)
0.65 (0.22, 1.98)
13.18
W C You (2001) *
0.75 (0.31, 1.78)
18.24
Mahmoud Zureik (2004)
1.42 (0.58, 3.50)
17.37
Serge Hercberg (2004)
0.97 (0.78, 1.21)
42.50
Subtotal (I-squared = 39.6%, p = 0.142)
0.78 (0.49, 1.26)
100.00
Selenium dose = 200µg/day
Saverio Stranges (2006)
1.03 (0.78, 1.37)
35.36
Scott M. Lippman (2009)
1.02 (0.92, 1.13)
50.74
Urban Alehagen (2012)
0.45 (0.23, 0.87)
13.90
Subtotal (I-squared = 66.0%, p = 0.053)
0.91 (0.69, 1.21)
100.00
Overall (I-squared = 45.3%, p = 0.067)
0.91 (0.74, 1.10)
.
NOTE: Weights are from random effects analysis
.15
.25
.5
.75
1
2
3
4

Figure 4.

Supplemental materials

Full title: Selenium and Cardiovascular Disease: A Meta-Analysis Assessing the Discrepancies between Observational and Randomized Trial Evidence

Supplemental tables

Supplemental table 1. Study characteristics of 11 prospective observational studies (16 independent studies) of blood (plasma/serum/erythrocyte) or toenail Selenium Levels and CVDs events

Supplemental table 2. Study characteristics of 16 RCTs of selenium supplementation and CVDs events

Supplemental figure

Supplemental Figure 1. Dose-response relation between baseline concentrations of selenium and the risk of CVDs in 16 independent prospective studies. The relation is fitted by using the restricted cubic spline.

Supplemental Figure 2. Dose- and Duration-dependent changes of selenium concentrations in 10 independent RCTs. Trial data were graphically shown on mean changes in plasma selenium concentrations (μg/L) after selenium treatment vs. placebo by two different supplemental doses (100 and 200μg/day) from 1 week to 48 months.

Supplemental Figure 3. Trial data on percentage changes of GPx activity in blood selenium levels after selenium treatment compared with baseline from 1 to 48 weeks. The smooth curve represents median of percentage changes of GPx activity at baseline, 1, 4, 6, 12, 24 and 48 weeks.

Supplemental table 1. Study characteristics of 11 prospective observational studies (16 independent studies) of blood (plasma/serum/erythrocyte) or toenail Selenium Levels and CVDs events

Author, Publication year	Source	Population	Design	Age, year	Follow-up years	N (cases/controls or participants, gender)	End Point	Main Outcome (Highest vs. Lowest)	
								Selenium ranges (median or mean); RR (95% CI)	Covariates adjusted in the full model
Jukka T. Salonen, 1982	Eastern Finland Heart Survey, Finland	Population-based	Cohort	35 - 59	7	Cases: 208 men and 75 women; Controls: 208 men and 75 women	CVD Mortality	49.5 vs. 34.5μg/L; 0.71 (0.2, 2.5)	History of angina pectoris, congestive heart disease and valvular heart defect, antihypertensive drug treatment, history of MI or AP in either parent, dietary saturated fats, intake of strong alcoholic beverages, and study area
Tatu A Miettinen, 1983	Eastern Finland Heart Survey, Finland	Population-based	Nested Case-control study	48 ± 1	5-7	Cases: 33 men Controls: 64 men	MI	93.84 vs. 51.97μg/L; 0.88 (0.49, 1.57)	NO
Jarmo Virtamo, 1985	National Death Certificate Register, Finland	Population-based	Cohort	55 - 74	6	Cases: 141 men Controls: 969 men	Coronary Heart Disease Mortality	30.51 vs. 82.29μg/L; 0.5 (0.25, 1)	Age and area
Jukka T. Salonen, 1985	Eastern Finland Heart Survey, Finland	Population-based	Cohort	30 - 64	5	Cases: 69 men and 23 women Control: 69 men and 23 women	Coronary Artery Disease Mortality	≥45 vs. < 45μg/L; 1.11 (0.43-3.33)	Intake of strong alcoholic beverages, days of work absenteeism, diabetes, history of myocardial infarction or angina pectoris in either parent, cardiovascular medication and study

									area
Jetmund Ringstad, et al, 1987	The Troms Ø Heart Study, Norwegian	Population-based	Nested Case-control study	28 - 54	6	Cases: 59 men Controls: 59 men	Myocardial Infarction	104.64 vs.130.34μg/L; 1.0 (0.43, 2.5)	NR
Frans J Kok, et al, 1987	Epidemiologic Prevention Study Zoetermeer (EPOZ-Study), Netherlands	Population-based	Nested Case-control study	37 - 87	9	Cases: 47 men and 37 women Controls: 94 men and 74 women	CVDs Death	164.6 vs. 141.35μg/L; 0.5 (0.2, 1.25)	gender, age, serum cholesterol, systolic and diastolic blood pressure, smoking, body mass index, week of blood collection, years of education, history of myocardial infarction, and history of stroke.
P. Suadicani, 1992	The Copenhagen Male Study, Denmark	Population-based	Cohort	53 - 74	3	Cases: 107 men Controls: 2893 men	Ischemic Heart Disease	108.22 vs. 64.24μg/L; 0.59 (0.40, 0.88)	serum cholesterol, smoking, social class, age
Simonetta Salvini,et al, 1995	The Physicians' Health Randomized Trial Study, USA	Physicians Population	Nested Case-control study	40 - 84	5	Cases: 251 men Controls: 251 men	Myocardial Infarction	136.84 vs. 94.81μg/L; 1.27 (0.71, 2.29)	NO
Jukka Mamiemi, 1998	Health survey with complete clinical evaluation, Finland	Population-based	Cohort	≥65	13	Cases: 78 men and 64 women Controls: 104 women and 98 women	CVD death	NA; 1.08 (0.68, 1.72)	NO
Wen-Qiang Wei, 2004	Nested study from the Nutrition Intervention Trial, China	Population-based	Nested Case-control study	40 - 69	15	Cases: 78 men and 38 women Controls: 530 men and 457 women	HD Mortality	86.79 vs. 52.07μg/L; 0.66 (0.41, 1.08)	Sex, age, cholesterol, smoking, drinking, and BMI, diastolic and systolic blood pressure.

N. Tasnime Akbaraly, 2005	EVA study, France	Population-based	Cohort	59 - 71	9	Cases: 22 Controls: 1268	CVD death	97.4 vs. 76.2μg/L; 0.82 (0.46, 1.45)	Sociodemographic characteristics, dietary habits, health, and cognitive factors.
Joachim Bleys, et al, 2008	The Third National Health and Nutrition Examination Survey (NHANES III), United States	Population-based	Cohort	20 - 90	12	Cases: 881 Control: 13006	Cardiovascular Mortality	136.92 vs. 110.78μg/L; 1.0 (0.81, 1.23)	Age, sex, race/ethnicity, education, annual family income, postmenopausal status for women, cigarette smoking, serum cotinine level, alcohol consumption, physical activity, body mass index, and vitamin and/or mineral supplement use
Charles B. Eaton et al, 2010	The Third National Health and Nutrition Examination Survey (NHANES III), United States	Population-based	Cohort	≥35	13.4	Cases: 1038 Controls: 9493	CHD Mortality	133.5 vs. 81μg/L; 0.87 (0.56, 1.33)	Age
Maria Wennberg, 2011	Northern Sweden Health and Disease Study (NSHDS), Sweden	Population-based	Nested Case-control study	30 - 77	13	Cases: 350 men and 150 women Controls: 350 men and 275 women	Myocardial Infarction	143.4 vs. 108.2μg/L; 1.0 (0.6, 1.69)	Apolipoprotein B/ apolipoprotein A-I, smoking, systolic blood pressure, diabetes, education, consumption of fruit and vegetable, wine, strong beer, and level of physical activity.
Kazuko Yoshizawa, 2003	Health Professionals Follow-up Study (HPFS) , USA	Health Professional Population	Nested Case-control study	40 - 75	6	Cases: 470 men Controls: 465 men	Coronary Heart Disease	1.1 vs. 0.71ng/g; 0.96 (0.63, 1.45)	Age and smoking
Swapnil Rajpathak, 2005	Health Professionals follow-up Study (HPFS), USA	Health Professional Population	Nested Case-control study	40 - 75	12	Cases: 202 men Controls: 361 men	CVD	1.2 vs. 0.76ng/g; 0.60 (0.36, 0.97)	Age

* NA, Not available, NR, Not reported

Supplemental table 2. Study characteristics of 16 RCTs of selenium supplementation and CVDs events

First author, year	Source	Sample size	Age, year	Selenium form (dose μg/d)	Selenium combination	Follow-up Years	Quality*	End Point
H. Korpela,1989	Finland Acute MI	Placebo 41 Treatment: 40	Placebo: 58 Treatment: 56	100μg/day Selenium yeast	No	6m	2	MI and cardiac death, selenium concentration
B. Kuklinski,1994	NR Acute MI	Placebo: 29 Treatment: 32	Treatment: 62 Placebo: 61	100μg/day Bio-selenium	Coenzyme Q10	12m	1	Death from re-infarction
B. Greg Brown, 2001	HDL-Atherosclerosis Treatment Study (HATS) Coronary disease patients	Placebo: 76 Treatment: 84	Male: < 63 Female: < 70	100μg/day NR	800 IU vitamin E, 1000 mg vitamin C, 25 mg natural β-carotene	3y	5	Death from coronary causes, nonfatal myocardial infarction, stroke, or revascularization for worsening ischemia
W C You &Mitchell H. Gail, 2001 1998	China Village residents	Male: 1753 Female: 1658	35 – 64	75μg/day Selenium yeast	800mg garlic, 4mg garlic oil, 500mg vitamin C, 200 IU vitamin E, 15mg β-carotene	39m	5	Cardiovascular deaths all-cause mortality
Serge Hercberg, et al, 2004	SU.VI.MAX Study, French Volunteers	Placebo: 6364 Treatment: 6377	Female: 35 - 60 Male: 45 - 60	100μg/day Selenium yeast	120 mg ascorbic acid, 30 mg vitamin E, 6 mg β-carotene, and 20 mg zinc	7.5y	5	Incidence of Ischemic CVD, overall mortality, selenium concentration
Mahmoud Zureik, 2004	SU.VI.MAX Study, French Volunteers	Placebo: 599 Control: 563	≥50	100μg/day Selenium yeast	120 mg vitamin C, 30 mg vitamin E, 6 mg beta carotene, and 20 mg zinc	7.2 ± 0.3y	4	CHD incidence
Saverio Stranges, 2006	NPC Trial, USA. Population free of CVDs	Male: 714 Female: 290	63.2	200μg/day High-selenium baker's yeast tablet	No	7.6y	5	CVD incidence, CVD mortality, all-cause mortality, stroke, MI (fatal and nonfatal MI) and CHD

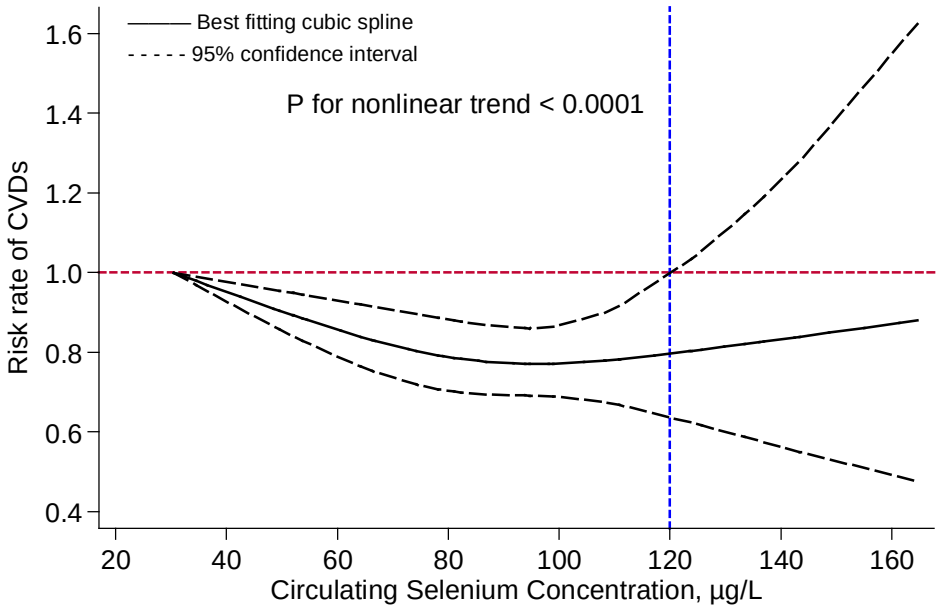
Renate Schnabel, 2008	SETCAP Study, Germany Coronary artery disease patients	Placebo: 132; Se 200: 132; Se 500: 128	66	200 and 500μg/day Sodium selenite	No	12w	5	Selenium concentration
Scott M. Lippman, 2009	SELECT, United States, Canada, and Puerto Rico Volunteers	Placebo: 8696 Treatment: 8752	≥50	200μg/day L-selenomethionine	No	7 -12y	5	Cardiovascular deaths, all-cause death and cardiovascular events (mortality and incidence), selenium
Margaret P. Rayman, 2011	PRECISE Pilot Study, United Kingdom Volunteers	Placebo: 107 Se 100: 123 Se 200:124 Se 300: 120	67.4 ± 4.1	100, 200 and 300μg/day High-selenium yeast	No	≥6m.	5	Selenium concentration
Urban Alehagen, 2012	NR Rural municipality inhabitants	Male: 225 Female: 218	76.2	200μg/day Organic selenium yeast	Coenzyme Q10	5y	5	CVD and all-cause mortality
Jody C Miller 2012	New Zealand Patients with coronary artery disease	Male: 138 Female: 117	38 – 90	100μg/day L-selenomethionine	No	12w	5	Selenium concentration
Wayne Chris Hawkes 2008	North American Healthy men	Male: 42	18 – 45	300μg/day High-Se Baker's yeast	No	48w	4	Plasma Se
Gitte Ravn-Haren 2008	Denmark Healthy male volunteers	Placebo: 20 Selenate: 20 Se-enriched yeast: 20 Se-enriched milk: 20	18 – 40	Selenate and Se-enriched yeast: 300μg/day	No	4w	4	Selenium concentration
P. V. Luoma 1985	Finland Healthy medical students volunteered	Male: 8 Female: 15	21 – 34	Selenium yeast tablets: 96μg/day	No	2w	4	Selenium concentration

James R. Marshall 2011	USA High-grade prostatic intraepithelial neoplasia patients	Placebo: 51 Treatment: 46	≥40	Selenomethionine 200μg/day	No	3y	4	Selenium concentration

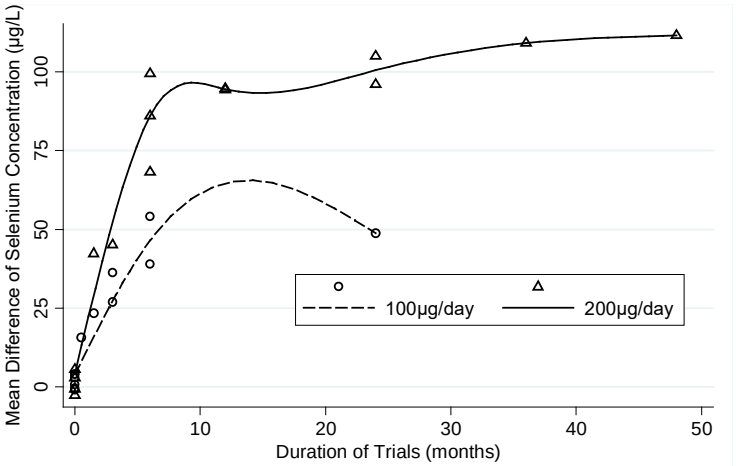
*The 5-point Jadad Score based on the description of randomization, double blinding and withdrawals. NA, not available. NR, not reported

Supplemental figure

Supplemental figure 1.



Supplemental figure 2.



Supplemental figure 3.