Title: Selenium and immune function: a systematic review and meta-analysis of experimental human studies

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Abbreviations:

CI: confidence interval

GPX: glutathione peroxidase

KD: Keshan disease

IQR: interquartile range

NK: natural killer

PICOS: Population, Intervention, Comparison, Outcome, Study design

PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses

SD: standard deviation

SE: standard error

SMD: standardized mean difference

Abstract

Background: Selenium is an essential trace element with both beneficial and detrimental effects on health depending on dose and chemical form. Currently, there is debate about recommendations for selenium supplementation as a public health measure to improve immune function and reduce infectious disease susceptibility.

Objectives: We performed a systematic review and meta-analysis of experimental studies assessing the effect of selenium supplementation on immunity-related outcomes in healthy people.

Methods: We undertook a search of published and unpublished studies in literature databases PubMed/MEDLINE, Embase and Clinicaltrials.gov up to October 17, 2022, and performed a meta-analysis comparing the effects on immunity-related outcomes between supplemented versus control arms. Whenever possible we assessed the nonlinear relation using a dose-response approach.

Results: Nine trials were included, five in North America, and four in Europe, with duration between 8-48 weeks and supplementation of both inorganic and organic selenium forms. Selenium supplementation did not substantially affect immunoglobulin or white blood cell levels, and the dose-response meta-analysis indicated that an increase in plasma selenium concentrations above 100 µg/L did not further increase IgA levels nor T-cells. An inverted U-shaped relation emerged for Natural killer (NK) cell count, with lower number of these cells both below and above 120 µg/L. The only beneficial effect of selenium supplementation was increased activity for NKlysis, but the available data did not permit dose-response analysis. Cytokine levels were substantially unaffected by selenium supplementation.

Conclusions: Although some of the data suggested beneficial effects of selenium supplementation on immune function, the overall picture appears to be inconsistent and heterogenous due to differences in trial duration and interventions, plus evidence of null and even detrimental effects. Overall, the evidence that we extracted from the literature in this systematic review does not support the need to

supplement selenium beyond the recommended dietary intake to obtain beneficial effects on immune function.

Keywords: selenium, immune function, infectious disease, experimental studies, systematic review, dose-response meta-analysis

PROSPERO (registration no. CRD42022312280)

Introduction

Selenium is an essential trace element that has a complex and intriguing relationship with human health, demonstrating both beneficial and detrimental effects, depending on the dose and on the chemical species (1). Selenocysteine is recognized as the 21st amino acid (2) and selenium is an essential component of 25 selenoproteins (3) which participate in a wide variety of physiological processes, including the regulation of antioxidant response to reactive oxygen species and other physiological properties (1, 2, 4). Levels of such functional biomarkers were used by various national and international bodies to suggest dietary recommendations for selenium intake (5-8), i.e. both average requirements and dietary reference intakes, ranging from 20 to 75 µg/day depending on the type and amount of selenium-induced proteomic response chosen (1). Some of these selenoproteins are enzymes that could also be involved in immune function, such as glutathione peroxidases (GPXs), thioredoxin reductases, iodothyronine deiodinases, methionine-R-sulfoxide reductase B1, and selenophosphate synthetase 2 (9). For this reason and for some laboratory studies (10), selenium deficiency has been suggested as having a negative impact on defense against infectious diseases.

In particular, the association between selenium deficiency and adverse health outcomes in humans was originally proposed with the identification of Keshan disease (KD). This disorder is characterized by a severe cardiomyopathy, and it was first recorded in 1935 where it was mainly found in parts of the country having as common features a low selenium concentrations in soils and foods locally produced (11). However, some epidemiological hallmarks of KD could not be explained solely on the basis of selenium deficiency. In particular, seasonal fluctuations in KD incidence suggested involvement of an infectious agent (1, 11). Coxsackie virus was, in fact, detected in the myocardium of KD patients (12), and studies in mice exposed to Coxsackievirus showed that host nutritional deficiency led to viral genome mutations which rendered benign viruses highly virulent (13). In addition, other animal and in vitro studies indicated that selenium is able to inhibit

the viral replication of Coxsackievirus (14, 15). These studies illustrate the complexity of selenium interactions in the body and also indicates that specific host nutritional status can alter viral genotype. In relation to this, it should be noted that other trace elements and vitamins may be implicated in the etiology of KD in relation to both nutritional status and viral infection (16, 17), as well as genetic factors such as genetic polymorphisms, including of *GPX* genes (11).

Overall, interest in the relationship between selenium and immune system/function has been increased over the past years (18, 19). Results from cell and animal models have demonstrated that humoral (adaptive) immunity, such as activation and functions of T and B cells, is affected by the level of selenium exposure; cell-mediated (innate) immunity, including inflammatory signaling capacity and anti-pathogen activities of macrophages, is also influenced by selenium (9). However, there are conflicting reports from human trials designed to demonstrate the benefits of selenium supplementation to boost immunity against bacterial and viral pathogens. In view of the current interest in the role of nutrition in the immune system (20) we attempted to estimate the intake of selenium that is associated with optimal immune function. We undertook a systematic review of selenium and infectious disease susceptibility, focusing on data extracted from the studies providing the highest level of evidence, namely randomized controlled trials of selenium supplementation and measures of immune function, performing a dose-response meta-analysis whenever possible.

Methods

After registering the protocol in PROSPERO (registration no. CRD42022312280), we carried out a systematic review according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (21).

Study identification and selection

We conducted a search of online literature databases (PubMed/MEDLINE and EMBASE) and ClinicalTrials.gov up to October 17, 2022, for experimental human studies assessing the association between selenium status and infectious disease susceptibility. We defined the Population, Intervention, Comparison, Outcome, Study design (PICOS) statement as "In healthy adults, what is the susceptibility to infectious diseases in relation to selenium status when assessed in experimental studies investigating the effects of selenium supplementation?". We used as search keywords terms related to 'humans', 'selenium' or 'selenium supplementation', 'infectious disease', 'immune system' or 'immunity', and 'trial' or 'clinical trial'. Details of database searches are reported in Supplementary Table 1. Inclusion criteria were: being an epidemiological study in healthy humans, selenium being the only difference between experimental and control group, and the outcome related to infectious disease susceptibility or immune system. We excluded nonexperimental studies, case reports, reviews and commentaries.

Risk of bias assessment

We performed an assessment of the risk of bias of the included studies using the RoB 2.0 tool of the Cochrane collaboration (22) using the subsequent five domains: (1) bias arising from randomization process; (2) bias due to deviations from the intended interventions investigating the effect of assignment to intervention(i.e. intention-to-treat-analysis); (3) missing outcome data; (4) bias in measurement of the outcome; (5) bias in selection of the reported result. For each domain the judgement can be low risk, some concerns, and high risk. Overall, we judged a study at 'low risk' of bias if it had a low risk in all domains, while we judged as having 'some concerns' when at least one domain was classified as such, but none was at high risk. A study was considered at high risk of bias if there was high risk in at least one domain.

Data extraction

We extracted the following data from included reports: first author name, publication year, country, type, duration of the trial, study population and characteristics, trial design and characteristics (randomization, blinding), dose and duration of selenium supplements, selenium concentrations before and after the intervention and difference between intervention and control group at the end of the intervention, outcomes of interests related to infectious disease and immune function. When data were mentioned but not explicitly reported or only partially available e.g. only in figures and not in a tabular way, we sought to contact study investigators to retrieve the raw data. Whenever possible we extracted mean or median values along with standard deviation (SD), standard error (SE) or interquartile range (IQR). When data were available only from figures, we sought to extract mean levels along with SD or SE from figures. In relation to data extraction, we systematically tried to contact study authors to request data when those available in the publication were not enough to include the study in the dose-response meta-analysis. However, authors could not be reached for two studies since they were no longer working (23, 24). For one study, we did not get any answer despite the availability of email address from recent papers (25) and for another study, the corresponding author confirmed that original data were no longer available (26). Finally, for one study, information was available about study design (e.g. randomization, blinding), but raw data was no longer available (27), but we were able to retrieve original data from a later study for use in the meta-analysis (28). In order to perform quantitative analysis, in five out of nine studies, we sought to use data reported in figures (23-27). For this meta-analysis we extracted findings for a specific endpoint when at least two studies for that endpoint were available. When three or more studies for an endpoint were available, we also extracted data about plasma selenium concentrations at the end of the intervention period (20, 25, 27).

Data analysis

We performed a meta-analysis comparing higher versus the lowest category i.e. treated versus control group. We did this through computation of Hedges's g standardized mean differences (SMD) along with their 95% confidence interval (CI), due to heterogeneity in units of measurements of outcomes when at least two studies were available for each specific outcome. In addition, whenever possible, we performed dose-response meta-analysis of SMD between selenium levels and parameters of immune function. We used the one-stage methodology (29, 30), an approach for implementation of dose-response meta-analysis based on a weighted mixed effects model and using cubic splines that enables the pooling of results from all studies when at least two levels of exposure are available, as implemented in other fields (31, 32). Having no specific parametric assumption about the shape of the association, we used restricted cubic splines with 3 knots at fixed percentiles (10th, 50th, and 90th) to investigate such association taking into account both difference in plasma selenium concentrations between the treatment and control groups and final plasma selenium concentrations at the end of the intervention. For each spline, we used as reference dose the median value of the set of studies alternatively considered in each specific analysis. All analyses were carried out by using 'meta' and 'drmeta' routines of Stata statistical software (Stata 17.0-SE 2021, StataCorp LLC, College Station, TX) for all data analysis. For all data analyses we did not use null hypothesis testing and p-value cutpoints, in accordance with the American Statistician Association guidelines (33) and recent literature in the field (34, 35).

Results

Figure 1 shows the flow-chart for study retrieval and selection. After removal of duplicates we identified 331 records in online databases, and we further excluded 258 records based on title/abstract

screening. After full-text evaluation, we eventually included in the final analysis 9 studies (23-28, 36-38), one of which was retrieved through citation chasing (37). Reasons for exclusion after full-text evaluation are reported in detail in Supplementary Table 2.

Overall, characteristics of the nine included studies are reported in Table 1. Five studies were carried out in North America/US (23, 24, 26, 37, 38), two in the UK (27, 28), one in Belgium (25) and one in Finland (36). Overall, they included 370 participants (220 in selenium-treatment groups and 150 in the control groups) with ages ranging from 18-64 years in all but two studies; one was undertaken in elderly institutionalized subjects aged ≥65 years (25) and one in subjects aged 57-84 years (26). Three studies recruited only male participants (36-38), while others six studies both males and females, though none reporting sex-stratified analysis. Duration of the trials ranged from 8 to 48 weeks. Selenium supplementation occurred in the inorganic form using sodium selenite in three trials (23, 24, 27). The intervention doses varied with 50 and 100 μg/day used in one trial (27), and 200 μg/day in the remaining two trials (23, 24). Conversely, organic selenium was administered in the form of selenized yeast in five trials (25, 26, 28, 36, 38). Doses varied from 50 up to 400 μg/day, with some trials having two or more intervention groups at increasing doses of selenium. In addition, two trials also used Se-rich foods to increase selenium intake in the intervention groups. Specifically, one trial administered wheat toast made with Se-rich flour (36) and the other Se-enriched onions (28). Finally, one trial administered to study participants Se-low and Se-high diets in the control and intervention groups directly providing three daily meals made with foods, namely rice and beef staples, from different geographical origins with very low or very high soil selenium (37).

All studies measured plasma concentrations of selenium before and at the end of the intervention. Baseline plasma concentrations were generally similar in the control and intervention groups, ranging between 70 to 118 μg/L (mean 110 μg/L) and from 66 to 142 μg/L (mean 103 μg/L), respectively. Selenium concentrations at the end of the interventions were systematically raised (mean in the

selenium-supplemented group 144 µg/L, range 92-228 µg/L) but not in the placebo group (mean 108 µg/L, range 72-153 µg/L). However, in two trials, plasma concentrations of selenium did not increase in the intervention groups and were substantially similar to those of control groups (23, 24). Finally, in one trial (26), selenium supplementation did increase the baseline selenium concentrations (from 129 to 142 µg/L), while the control group showed constantly higher concentrations at both baseline and at the end of the trial (155 vs. 154 µg/L).

Results of risk of bias assessment are reported in Supplementary Table 3. Most of the included trials were judged at 'low risk' of bias. One study was judged as having 'some concerns' due to the single-blind design, although no deviations from the intended intervention were detected (26). Two additional trials (23, 24) were classified as 'some concerns' in the randomization process due to the lack of reporting of detailed characteristics of recruited subjects at baseline, hampering the evaluation for this item. In addition, these two trials were judged as 'high risk' of bias due to deviations from the intended interventions since no information about blinding were reported. Furthermore, participants had substantially comparable plasma selenium concentrations in the intervention and control arms at the end of the trial, thus raising questions about the reliability of study findings. For these reasons, they were judged as having an overall 'high risk' of bias.

In **Figure 2**, the meta-analysis of studies assessing Ig levels showed small to null increase for all Ig types due to selenium supplementation (IgA: SMD = 0.13; 95% CI -0.16, 0.42; IgG: SMD = 0.14; 95% CI -0.97, 1.25; IgM: SMD = 0.09; 95% CI -0.50, 0.67). All studies were at low risk of bias and used organic selenium forms, i.e. selenized yeast, through diet or foods rich in selenium. In **Figure 3** we reported the dose-response meta-analysis (implemented for IgA levels only). When looking at plasma selenium difference between treatment and control arms, selenium increase above the median value (40 µg/L) seems to be associated with higher IgA levels. When looking at final plasma

selenium concentrations at the end of the trials, such increases can be detected up till 110 µg/L, while further increases in selenium are not associated with any change in IgA levels.

Figure 4 shows the SMD for lymphocyte overall levels and subtypes (B-cells, T-cells and T CD4+ and CD8+ cells). Based on only two studies, selenium supplementation seems to be associated with a decrease in total lymphocyte levels (SMD=-0.25; 95% CI -0.77, 0.27), while it had no effect on B-cells. Conversely, a slight increase on T-cells was noted, though it is very imprecise and when only studies at low risk of bias were considered the increase was less (Supplementary Figure 1).

Figure 5 presents the dose-response analysis for T-cells and T CD8+ cells for which a sufficient number of studies was available. When considering both difference and final selenium concentrations, an increase can be noted until approximately +10 µg/L and 110 µg/L respectively, above which a plateau is reached and there is a null increase in total and T CD8+ cells. We observed comparable results when considering studies at low risk of bias only (Supplementary Figure 2).

Figure 6 presents the SMD for Natural killer (NK) cell levels and activity, with inverse to null effects on NK cells (SMD=-0.14, 95% CI -0.49, 0.21), but increases in NK lysis (SMD=0.48, 95% CI 0.13, 0.82). Results are comparable when considering studies at low risk of bias (Supplementary Figure 3). In the dose-response analysis for NK cells, increases in plasma selenium seems to be associated with an increase in NK cells until 140 µg/L, but with further increase an inverse relation can be noted with a decrease in NK cells (**Figure 7**). A similar pattern is suggested when looking at differences in selenium concentrations, especially when studies at low risk of bias are considered (Supplementary Figure 4).

Supplementary Figure 5 presents SMD for expression or IL-2 receptors reported in two studies indicating no effect of selenium supplementation (either in the form of organic or inorganic selenium), with the same results after exclusion of one study at high risk of bias (Supplementary Figure 6). Supplementary Figure 7 shows the effect of selenium supplementation on cytokine levels,

223 namely IL-10 and IFN-gamma from two studies, both at low risk of bias, suggesting a slight though
224 imprecise increase (IL-10: SMD=0.07, 95% CI -0.18, 0.33 and IFN-gamma: SMD=0.20, 95% CI -
225 0.05, 0.45). Supplementary Figure 8 shows results of lymphocyte proliferation in either absence or
226 presence of external stimuli (e.g. different mitogens or vaccination) as assessed through total
227 lymphocyte proliferation in all but one study assessing T-cell proliferation (28). All studies are at low
228 risk of bias. Overall, lymphocyte proliferation seems to be unaffected by selenium supplementation.
229 For the analysis including IL-2 receptor, cytokine levels and lymphocyte proliferation, we could not
230 perform dose-response meta-analysis due to the limited number of studies.
231
232 **Discussion**
233 This review, to the best of our knowledge, the first to meta-analyze human trials on selenium
234 supplementation and immunological endpoints using a dose-response approach, provides little
235 evidence of beneficial effects of selenium supplementation on immune function as assessed through
236 the evaluation of levels of immunoglobulins, and white blood cells, particularly lymphocytes and NK
237 cells. In particular, the dose-response meta-analysis indicates that an increase in plasma selenium
238 concentration above 100 µg/L does not further increase IgA levels nor T-cells, as seen at lower
239 levels, suggesting the occurrence of a possible beneficial effect that corresponds to an intake of 70
240 µg/day, namely the Dietary Reference Value (DRV) for selenium (5), using a conversion factor of
241 1.5 as previously suggested (39). The pattern of association becomes more complex regarding NK
242 cells count, for which an inverted U-shaped relation emerged, with lower numbers of these cells both
243 below and above 120 µg/L (80 µg/day). Such a U-shaped pattern is not unusual for a nutrient,
244 considering that both levels of too low and too high exposure may induce adverse effects. The only
245 beneficial effect of selenium emerged for NK lysis, but data did not allow us to test any dose-
246 response relation or the possible presence of a U-shaped curve. The dose-response curve gave an

indication that increased selenium intake at the lower range of the selenium intake tested in the trials was beneficial, but that achieving intakes above the DRV Adequate Intake value of around 70 µg/day, as implied by final blood selenium concentrations of 110 µg/L, does not yield any further beneficial effect. It should also be noted that a recent observational study comparing the effects on IgG against SARS-CoV-2 in subjects with different intakes of selenium and/or habitual selenium supplementation found no effect of the selenium status on this parameter, which is consistent with the results of the trials (40).

Our findings also do not demonstrate that selenium supplementation influences cytokine levels such as IL-10, IFN-gamma, nor IL-2 receptor, contrary to earlier expectations and the working hypothesis driving the trials (18). Furthermore, results relating to lymphocyte proliferation due to mitogen exposure indicated that selenium supplementation induced, if anything, adverse effects depending on the selenium dose and the mitogen used, though such a relationship could not be investigated through a dose-response approach since the number of relevant studies was low. Previous animal and *in vitro* studies suggested that selenium may have immunomodulatory effects, including lymphocyte proliferation, antibody concentrations, and cytokine expression and reactivity (2, 18), as well as regulation of selenoprotein expression in T-cells (41). However, evidence in human is still controversial and limited evidence has been provided by human studies (42, 43).

It should be noted that the selenium status in the general population appreciably varies across countries. For example, the US selenium exposure tends to be higher than that in other Western countries, particularly European ones. According to the NHANES surveys, serum selenium levels in the US population are generally 130-140 µg/L (44-46), corresponding to a selenium intake of around 86-93 µg/day (1, 39). Conversely, selenium levels in the European populations are somewhat lower, ranging from 50-120 µg/L (5, 47, 48). For these reasons and according to our findings, the intake of selenium in these population can be considered adequate, with no need to increase selenium through

supplementation with reference to improve immune function. With regard to the presence of U-shape pattern with immune function, such an occurrence is not entirely unexpected for selenium, as more generally for micronutrients and other nutrients, for which adverse effects at both low and high exposure levels may occur (49). Specifically for selenium, a narrow safe range of intake has been already suggested for other health outcomes such as type 2 diabetes. For the latter disease, an excess risk has been identified in human experimental studies where 200 µg/day of selenium supplementation was used in the intervention arms (50), and for increases of blood selenium concentrations approximately above 90 µg/L in nonexperimental studies (32). Overall, our findings seem to confirm that selenium exposure can be considered beneficial for the immune system until a plasma selenium concentration of approximately 100 µg/L, while higher levels may be associated to null or adverse effects.

Recently, interest has been raised about the possible relationship between selenium intake and COVID-19, under the hypothesis that an impaired selenium status could favor SARS-CoV-2 infection and spread and COVID-19 severity (51). However, experimental evidence from human trials is still lacking as no trials have been performed so far (52), and some evidence from two recent Mendelian randomization studies investigating this issue were unable to confirm this possible relationship (53, 54). Similarly, a lack of association was noted for another disease of high public health relevance, HIV infection: despite some evidence from observational studies of higher risk of infection in subjects with low selenium status (43), findings from experimental studies suggested that increasing selenium intake might delay CD4+ cell decline but not induce viral suppression (55, 56).

Our review has limitations that must be acknowledged. Firstly the low number of trials investigating some of the outcomes hampered the implementation of dose-response meta-analysis by restricting the range of exposure suitable for analysis and decreasing the statistical precision of the estimates, and for many endpoints (e.g., IgG levels, NK lysis, lymphocyte proliferation, etc.) even

precluded such analysis. For some of the included studies we extracted numerical data from figures in order to perform quantitative analysis whenever possible, thus possibly inducing some additional amount of imprecision of the individual estimates. Secondly, some of the studies we retrieved and included in the analysis were affected by methodological flaws and potential severe sources of bias, including lack of randomization, blinding and compliance with the intervention, thus considerably reducing the reliability of the results. With respect to selection bias, most of the studies excluded participants with chronic diseases, especially cancer, cardiovascular diseases or diabetes) and some also included cutoff levels of body mass index in order to exclude subjects with obesity. However, we cannot entirely rule out that some subjects with metabolic disorders could still have been recruited in such studies, somehow limiting the generalization of our findings to the general healthy population. However, all but two trials included subjects younger than 65 years, and those including older participants found consistent results to other studies, thus strengthening the reliability of our analysis.

The strength of this review is that it is the first that systematically addressed the experimental effects of selenium supplementation in humans with reference to immunological endpoints using whenever possible a dose-response approach to assess the level of selenium exposure that could be associated with beneficial or adverse effects on immune system.

Overall, the assessment and the meta-analysis of the experimental human studies investigating the immunological effects of selenium administration yielded limited evidence of beneficial effects of this intervention, and indicated that such beneficial effects were present only in subjects with a low selenium status and occurred only up to intakes of around 70 µg/day, whereas higher intakes were associated with null or even adverse effects.

Author Contributions

SF-T conceived the study with MV and TF. All authors retrieved and selected relevant papers. TF extracted data with supervision of SF-T and MV. TF performed statistical analysis and interpreted them with SF-T and MV. TF prepared the first draft manuscript with substantial contribution of other authors. All authors revised and approved the final manuscript.

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Table 1. Characteristics of included studies.

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (µg/L)	Outcomes	Results
Arvilommi 1983 (36)	Finland	40 heathy men aged 36-50 years, with no history of cardiovascular, pulmonary and psychiatric diseases, with low Se status (<70 µg/L)	yes	DB	11w Sep1981 - Dec1981	Se-yeast (200 µg/day) + Se-rich "zwieback-style" toast (made with Se-rich wheat flour)	20 C 20 T (10+10)	Mean ± SD Baseline[3]: C: 70 ± 9 T: 70 ± 10 End of trial: C: 74 ± 9 T: 169 ± 19	Specific response: Ig levels + plaque forming + lymphokine synthesis + proliferation after mitogen stimulation Aspecific response: phagocytosis + intracellular killing + chemiotaxis	The difference in the Se status was not reflected in changes in any test for specific immune response (antibody formation, lymphokine synthesis or proliferative response against different mitogens. About a specific response, no substantial difference for phagocytosis but higher (+9.4%) intracellular killing in high-Se group were found. All at the end of the trial, no baseline reported **Ig concentrations in the supernatants of PWM-stimulated lymphocyte cultures (ng/mL)** IgG 1090 vs. 2180 IgM 5030 vs. 5740 IgA 517 vs. 887 **Plaque forming cells/10^6 viable cells** IgG 19100 vs. 16700 IgM 14900 vs. 11500 IgA 11300 vs. 12100 **Lymphokine synthesis (migration index)** 0.50 vs. 0.50 **Proliferative response (count per minute)** Control 810 vs. 1010 PHA 57600 vs. 57800 ConA 34800 vs. 32100 **Phagocytosis of *S. aureus* (CFU/104 granulocytes)** 2870 vs. 3220 **Number of ingested bacteria viable after 1h** 570 vs. 460 **Killing (% of ingested bacteria)** 77.2 vs. 85.2 **Leukotriene B4** 9690 vs. 8610
Broome 2004 (27)	UK, Liverpool	66 (M/F: 33/33) healthy nonsmoking subjects aged 20-47 years not taking	yes[4]	DB	15w (1999-2000)	Sodium selenite (50 or 100 µg/day)	22 C 22 T50 22 T100	Mean Baseline: C: 78.96 T50: 78.17 T100: 82.12 End of trial: C: 83.5	Specific response: cell-mediated immune response to vaccination with T cells	Assessment of specific cell-mediated immune response to vaccination: whole blood was stimulated in vitro with poliovirus antigen derived from the same live attenuated poliovirus vaccine given in vivo was assessed at 0, 7, 14 and 21 days after vaccination.

		medications with low Se status (<94.75 µg/L)						T50: 91.7 T100: 103.3 (from figure)	and poliovirus antibody production and detection rate Aspecific response: cytokine levels	**T lymphocyte** (CD3+) proliferative response higher at day 7 for Se groups, higher for placebo at day 14 but similar at day 21. Subsets of T cells (Total, CD4+, CD8+) and NK cytotoxicity at the end of the trial (day 21) showed increased cells in Se groups, specially CD8+. Similarly, NK cytotoxicity increased with increasing Se compared to placebo (data reported as percentages in figures) **Cytokine response** (IFN-gamma, IL-2, IL-4, IL-10). All but IL-4 increased after vaccination after 21 days: IL-2 in a dose-response manner, IL-10 similar between placebo and T100, higher in T50; IFN-gamma similar in placebo and T50, higher in T100. **Poliovirus antibody production** all increased, but no differences reported **Poliovirus detection rate** in feces was lower in Se-treated compared to placebo, indicating more rapid clearance. Also, mutations in the poliovirus were detected with much higher rate in the placebo group compared to Se-treated groups.
Hawkes 2001 (37)	US, California	11 healthy men aged 26-45 with weight for height lower than 125% of ideal and without chronic diseases or medication use	yes	DB	99d	Low-Se (13 µg/day) and high-Se diet (297 µg/day) due to origin of rice and beef staples	6 C 5 T	Mean ± SD Baseline: Low-Se: 117.65 ± 7.9 High-Se: 105.81 ± 18.95 End of trial: Low-Se: 72.4 ± 9.5 High-Se: 187 ± 23	Specific response: Ig levels + WBC levels (all and subpopulation) + mitogen response + secondary response (with influenza A and B, and diphtheria) + DHT skin response Aspecific response: Complement components + NK cell number and activity	BMI: Low-Se: 22.8 ± 3.3, range: 19-27 High-Se: 23.3 ± 4.4 range: 18-29 IgA and IgG substantially unaffected, IgM declined in both, slightly more in high-Se diet, C4 declined in both groups. Diphteria titre for secondary response showed to be higher in high-Se group at the end of the trial. WBC were 5% decreased in high-Se diet and 10% increased in low-Se diet; lymphocytes both increased; granulocytes 9% decreased in high-Se and 12% increase in low-Se. WBC subpopulation noted a tendency for a higher increase in high-Se for T suppressor, cytotoxic T and activated T cells. No effect after mitogen exposure was noted. No effect on DHS skin responses to total diameter and number of indurations. **Ig levels (baseline vs. final in low and high-Se diets; mg/dL)** IgA 260 vs. 260 low-Se/217 vs. 204 high-Se IgG 1086 vs. 1144 low-Se/1025 vs. 962 high-Se IgM 132 vs. 123 low-Se/101 vs. 89 high-Se

Complement components (mg/dL)
C3 112 vs. 112 low-Se/107 vs. 109 high-Se
C4 23.8 vs. 20.5 low-Se/20.7 vs. 18.7 high-Se

Influenza A and B, and Diphtheria titre comparison for secondary response; mg/dL
Influenza A and B showed similar levels of secondary response at the end of the trial (no baseline tested).
Diphteria: no at difference at baseline (1500 vs 14100 low-Se /2100 vs 15400 high-Se), but higher levels in high-Se group (14700 vs 16600 Se-low/12400 vs 23600 Se-high).

WBC (baseline vs final in low and high-Se diets ; thou/cu mm):
WBC 4.1 vs. 4.5 low-Se/6.1 vs. 5.8 Se-high-Se
Lymphocytes 1.66 vs. 1.78 low-Se/2.04 vs. 2.14 high-Se
Granulocytes 2.08 vs. 2.3 low-Se/3.61 vs. 3.3 high-Se

WBC subpopulation in 10^6/L:
B-cells (CD19+) 222 vs. 251 low-Se/307 vs. 294 high-Se
T-cells (CD3+) 1177 vs. 1290 low-Se/1502 vs. 1582 high-Se
T helper (CD4+) 715 vs. 791 low-Se/ 928 vs. 950 high-Se
T suppressor (CD8+) 415 vs. 446 low-Se/498 vs. 593 high-Se
NK cells 218 vs. 196 low-Se/201 vs. 261 high-Se
Cytotoxic T 14 vs. 7.8 low-Se/40 vs. 50 high-Se
Activated T 101 vs. 95 low-Se/262 vs. 322 high-Se
NK activity (% lisis) 44 vs. 42 low-Se/45 vs. 53 high-Se

Mitogen exposure in vitro a thymidine incorporation into cellular DNA as Bp/1000 cells
Control 0.044 vs. 0.037 low-Se/0.046 vs. 0.036 high-Se
PHA 5 10.0 vs 11.5 low-Se/9.7 vs. 10.8 high-Se
PHA 10 13.0 vs. 13.5 low-Se/12.0 vs 11.7 high-Se
ConA 10 4.9 vs 7.0 low-Se/4.2 vs. 5.7 high-Se
ConA 20 5.8 vs. 7.9 low-Se/4.8 vs. 6.8 high-Se
PWM 1 3.8 vs. 6.2 low-Se/3.3 vs. 5.3 high-Se
PWM 2 4.5 vs. 6.8 low-Se/3.8 vs. 5.8 high-Se

Hawkes 2009 (38)	USA, California	42 healthy nonsmoking men aged 18-45 years with self-reported absence of diseases, clinically normal blood count and blood chemistry, and without obesity	yes	DB	48w	Low Se vs. High-Se (300 µg/day selenized yeast)	20 C 22 T	Mean ± SD Baseline: Low-Se: 146 ± 19 High-Se: 142 ± 19 End of trial: Low-Se: 141 ± 18 High-Se: 228 ± 63	Specific response: WBC levels+ DHT Aspecific response: NK cell number	**DHT skin response** assessed with total diameter and number of indurations at 48h and 72h to seven antigens: tuberculin purified-protein derivative; mumps; tetanus toxoid; candida; trichophyton; streptokinase streptase; coccidioidin. No effect of Se. BMI: Low-Se: 24.6 ± 3.0, range 18.9–29.6 High-Se: 23.5 ± 2.2, range 19.7–27.3 Se did not affect total lymphocytes B cells, T cells, CD4, CD8, but NK cells increased in low-Se diet only as well as both T cells an NK cells expressing IL2 receptor. DHT skin response decrease by 57% in low-Se diet while decreased approximately 20-25% in high-Se diet **WBC levels (cells/µL whole blood)** Lymphocytes 1862 vs. 1841 low-Se/1883 vs. 1709 high-Se B-cells (CD19+) 322 vs. 286 low-Se/289 vs. 293 high-Se T-cells (CD3+)1399 vs. 1386 low-Se/1399 vs. 1269 high-Se T-helper (CD4+) 858 vs. 784 low-Se/822 vs. 755 high-Se T-suppressor/cytotoxic (CD8+) 476 vs. 457 low-Se/493 vs. 415 high-Se NK cells 270 vs. 337 low-Se/352 vs. 304 high-Se T-cells+IL2R 4.70 vs. 6.81 low-Se/5.40 vs. 5.40 high-Se NK cells+IL2R 1.48 vs. 1.99 low-Se/1.61 vs. 1.08 high-Se
Ivory 2017 (28)	UK, Norfolk	119 (M/F: 54/65) healthy subjects aged	yes	DB	12w	Group SeY: selenized yeast (Se methionine 50, 100 or 200 µg/day)	20 C 20 T50 21 T100 23 T200	Mean ± SD[5] Baseline SeY C: 92.0 (11.9)	Specific response: Proliferating T-cells after	**DHT skin response** assessed with total diameter and number of indurations at 48h and 72h to five antigens: tuberculin purified-protein derivative; mumps; tetanus toxoid; candida; trichophyton). DHT response decrease by 57% in low-Se while decreased approximately 20-25% in high-Se. Response to all five specific antigens decreased from baseline in both low and high-Se groups, but not for tetanus toxoid (unchanged in low-Se) and trychophyton (increased in high-Se). BMI: 26 ± 0.54 Evaluation of immune response after flu vaccination showed an inverse U-shaped association with Se supplementation, with higher T-cell proliferation in

		50-64 years with normal hematology, blood chemistry, blood pressure levels and BMI >18.5 and <35 with low Se status (Se <110 µg/L)				Group SeO: Se-rich onions with 50 Se µg/day	17 CO 18 TO	T50: 92.2± 13.3 T100: 98.6 ± 10.5 T200: 99.1 ± 9.3 Week 10 SeY: C: 93.7 ± 16.5 T50: 118.3 ± 13.1 T100: 152.0 ± 24.3 T200: 177.4 ± 26.3 Baseline SeO: CO: 93.3 ± 11.5 TO: 97.6 ± 11.5 Week 10 SeO: CO: 94.2 ± 15.0 TO: 106.0 ± 11.9	flu vaccination +cytolytic granules Aspecific response: NK cells + cytokines levels	group treated with 100 µg Se/day compared to those treated with both 50 and 200 µg Se/day at week 12. Similarly, cytolytic granules were lower in the group treated with 200 µg Se/day compared to all other SeY groups, while in the SeO group, Granzyme B levels were higher in the Se-rich onion group. Cytotoxic cells levels were not affected by Se supplementation. **Proliferating T cells at week 10 and week 12 (before and after flu vaccination at week 11)** Proliferation of T cells was similar between baseline and week 10 in all groups. T cells increased with a dose-response effect in Se-supplemented groups with 50 and 100 Se µg/day, but a lower increase occurred in 200 Se µg/day group compared to 100 Se µg/day after flu vaccination at week 12. **Cytolytic granules:** Lower granzyme B and perforin in 200 Se µg/day group compared to placebo at either week 10 or 12. Granzyme B levels higher in Se-rich onion group compared to non-rich onion. **Cytotoxic cells**: Se supplementation as either SeY or SeO did not have any effecs of number of any additional cytoxic cels subsent investigated (NK cells or Tctx-ADCC cells. **Cytokine levels:** Concentrations of IL-8, IL-10, IFN-gamma; TNF-alfa were assessed showing a dose-response increase for IL-8 and IL-10 after flu vaccination in SeY group, and for IL-8 and IFN-gamma in SeO group.
Kiremidjian-Schumacher 1994 (23)	US, New York University Dental Center	32 (M/F: 27/5) healthy subjects aged 24-36 divided in two sub-trials: - CL Group: 21 (M/F: 16/5) subjects assessing	yes[6]	NI	8w	Sodium selenite (200 µg/day)	10 C-CL 11 T-CL 5 C-NK 6 T-NK	Mean ± SE CL group: Baseline C: 133.5 ± 5.4 T: 130.3 ± 4.6 End of trial C: 133.6 ± 6.2 T: 138.5 ± 5.11 NK group: Baseline	Specific response: lymphocytes activity Aspecific response: NK cell activity	Average BMI of 25 in all groups but except in selenite treatment arm of NK group with BMI=22. Se levels substantially did not change after Se supplementation, raising issue about compliance. However, in both CL and KN groups, Se supplementation after 8w resulted in increased lytic activity in Raji tumor cells compared to baseline as well as control group. **CL group**

		cytotoxic lymphocytes - NK group: 11 men, assessing NK cells						C: 122.0 ± 4.0 T: 120.0 ± 7.0 End of trial C: 122.0 ± 10.0 T: 114.0 ± 4.0		Nonetheless, cytotoxic lytic activity of lymphocytes against Raji tumor cells was higher in the Se-treated group compared to control (45.6% vs 27.6%). In spite of similar cytotoxicity of activated lymphocytes between groups, number of lymphocytes required to kill a fixed number of tumor Raji cells was lower in the Se-treated group (-46.1% compared to placebo)
Peretz 1991 (25)	Belgium, Brussels	22 (M/F: 7/15) elderly institutionalized (≥1 y) subjects aged >65 years with no history of severe disabling diseases (e.g. cancer, cirrhosis, or diabetes)	NI	DB	6m	Selenized yeast (100 µg/day)	11 C 11 T	Mean ± SD Baseline: C: 69.5 ± 19.7 T: 66.3 ± 9.5 End of trial: C: 75.01 ± 19.74 T: 130.3 ± 34.7	Specific response: lymphocyte proliferation after mitogen exposure	**NK group** Increase in NK lytic activity against Raji tumor cells in Se-treated group compared to control (+108%) BMI: 28±6 in T, 24±6 in C Lymphocytes proliferation appeared to increase in the Se-treated group after stimulation with one mitogen, but not with others. Also, the response was much higher when using serum pooled from healthy donors than autologous serum for cell culture. **Lymphocyte proliferation:** Proliferation of lymphocytes cultured in pool serum of healthy donors, in response to PWM was higher in Se-treated group (+138% at 6m), while no effects was found in control group as well no effects in both groups can be noted for other mitogens (PHA and OKT3). When cultured in serum from subject itself (autologous serum) lymphocyte proliferation in response to PWM was lower compared to pool serum.
Roy 1994 (24)	New York University Dental Center	22 (M/F: 12/10) healthy people age 24-36 years	yes[6]	NI	8w	Sodium selenite (200 µg/day)	11 C 11 T	Mean ± SE Baseline: C: 128.6 ± 5.5 T: 130.1 ± 7.3 End of trial: C: 143.4 ± 7.4 T: 152.7 ± 6.8	Number of IL-2 receptor sites in peripheral mononuclear cells	Average BMI: 23.7 Se supplementation increased the number of cells expressing IL-2 receptor sites after PHA stimulation, by 43.8% after 48h but 19.1% after 72h.
Wood 2000 (26)	US, Southern Arizona	21 (M/F: 13/9) healthy nonsmoking people aged 57-84 years with no history of	yes	SB	6m	Selenized yeast (400 µg/day)	8 C 8 T	Mean ± SD Baseline: C: 155 ± 6.0 T: 129 ± 4.8 End of trial C: 153.8 T: 141.3	Specific response: Total T-cells and subgroups Aspecific response:	Se supplementation did not affect total WBC levels, while increased T-cells and particularly T helper CD4+. Conversely, NK cells showed similar levels in Se treated but NK activity resulted higher. **WBC counts** No changes in WBC differentials due to supplementation.

	chronic diseases (e.g. cancer, cardiovascular diseases or diabetes). Analyzed 16 (8C + 8T due to 22% attrition rate)	Total WBC and NK cells and activity	Total B-cells did not change in any group. Total T-cells increased >50% in Se treated, while decreased >20% in the control group. T helper CD4+ increased in all groups, with much higher (>150%) increase in Se treated group (T). **NK cells** NK cell levels were slightly higher in C but similar in T. NK activity was lower in C, and higher in T

Notes: BMI, body mass index; C, placebo/control group; ConA, concanavalin A; DHT, delayed-type hypersensitivity; Ig, immunoglobulins; PHA, phytohemagglutinin; PWM, pokeweed mitogen; OKT3, monoclonal anti-human T lymphocyte antibody; T, Se-treated group; [a]Male/female ratio reported whenever possible; [2]Duration in: days (d), weeks (w) or months (m); [3]Data reported in Levander et al., 1983 (57); [4]From personal communication with authors; [e]Data reported in previous report Hurst et al. 2010 (58); [6]Included sex, race, age, body weight, height, dietary habits, and history of vitamin intake and tobacco and alcohol in the randomization process.

Figure legends

Figure 1. Flow-chart for study identification in online databases and ClinicalTrials.gov registry.

Figure 2. Forest plot of Hedges's g standardized mean differences for Ig levels, all studies. The area of each grey square is proportional to the inverse of the variance of the estimated standardized mean difference (SMD) and horizontal lines represent the 95% confidence interval. Black diamonds represent point estimates of overall SMD for each group. The solid vertical line represents null effect, SMD=0. N= number of participants, SD=standard deviation Se/C= selenium levels (µg/L) in control group, Se/T= selenium levels (µg/L) in treatment group.

Figure 3. Dose-response meta-analysis of changes of IgA levels according to plasma selenium difference (A and C) and final levels (B and D) of plasma selenium (µg/L) between selenium supplemented and control groups at the end, all studies, N=3 (28, 36, 37). Solid black line represents the effect with variation of SMD (y-axis) according to the plasma selenium levels (x-axis). The curves are designed using restricted cubic spline method using three knots at fixed cutpoints (10th, 50th and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The grey area represents 95% confidence interval. The short-dashed line represents the null effect, SMD=0.

Figure 4. Forest plot of Hedges's g standardized mean differences for lymphocytes levels, all studies. The area of each grey square is proportional to the inverse of the variance of the estimated standardized mean difference (SMD) and horizontal lines represent the 95% confidence interval. Black diamonds represent point estimates of overall SMD for each group. The solid vertical line represents null effect, SMD=0. N= number of participants, SD=standard deviation, Se/C= selenium levels (µg/L) in control group, Se/T= selenium levels (µg/L) in treatment group.

Figure 5. Dose-response meta-analysis of changes in T-cells and T cytotoxic CD8+ levels according to plasma selenium difference (A and C) and final levels (B and D) of plasma selenium (µg/L) between selenium supplemented and control groups at the end of the trials, all studies, N=5 in A and

B (26-28, 37, 38), N=4 in C and D (27, 28, 37, 38). Solid black line represents the effect with variation of SMD (y-axis) according to the plasma selenium levels (x-axis). The curves are designed using restricted cubic spline method using three knots at fixed cutpoints (10th, 50th and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The grey area represents 95% confidence interval. The short-dashed line represents the null effect, SMD=0.

Figure 6. Forest plot of Hedges's g standardized mean differences for natural killer (NK) cells and lysis, all studies. The area of each grey square is proportional to the inverse of the variance of the estimated standardized mean difference (SMD) and horizontal lines represent the 95% confidence interval. Black diamonds represent point estimates of overall SMD for each group. The solid vertical line represents null effect, SMD=0. N= number of participants, SD=standard deviation, Se/C= selenium levels (µg/L) in control group, Se/T= selenium levels (µg/L) in treatment group.

Figure 7. Dose-response meta-analysis of changes in NK cell levels according to difference (A) and final (B) levels of plasma selenium levels (µg/mL) between selenium supplemented and control groups at the end of the trials, all studies, N=4 (26, 28, 37, 38). Solid black line represents the effect with variation of SMD (y-axis) according to the plasma selenium levels (x-axis). The curves are designed using restricted cubic spline method using three knots at fixed cutpoints (10th, 50th and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The grey area represents 95% confidence interval. The short-dashed line represents the null effect, SMD=0.