The American Journal of Clinical Nutrition 117 (2023) 93–110

The American Journal of CLINICAL NUTRITION

journal homepage: www.journals.elsevier.com/the-american-journal-of-clinical-nutrition

Original Research Article

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

Tommaso Filippini [1,2], Susan Fairweather-Tait [3,*], Marco Vinceti [1,4]

[1] *Environmental, Genetic and Nutritional Epidemiology Research Center (CREAGEN), Section of Public Health, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy;* [2] *School of Public Health, University of California Berkeley, Berkeley, CA, USA;* [3] *Norwich Medical School, University of East Anglia, Norwich, UK;* [4] *Department of Epidemiology, Boston University School of Public Health, Boston, MA, USA*

A B S T R A C T

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Methods: We undertook a search of published and unpublished studies in literature databases such as PubMed/MEDLINE, Embase, and clinicaltrials.gov up to 17 October, 2022, and performed a meta-analysis comparing the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

Results: 9 trials were included, 5 in North America, and 4 in Europe, with a duration between 8 and 48 weeks and supplementation of both inorganic and organic selenium forms. Selenium supplementation did not substantially affect immunoglobulin or white blood cell concentrations, 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 NK cell count, with a lower number of these cells both below and above 120 μg/L. The only beneficial effect of selenium supplementation was the increased activity for NK lysis, 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 heterogeneous 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.

This trial was registered at PROSPERO (CRD42022312280).

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

Introduction

Selenium is an essential trace element with a complex and intriguing relationship with human health, demonstrating both beneficial and detrimental effects, depending on the dose and 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], that is, both average requirements and dietary reference intakes, ranging from 20 to 75 μg/d 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

Abbreviations: GPX, glutathione peroxidase; KD, Keshan disease; PICOS, Population, Intervention, Comparison, Outcome, Study design; SMD, standardized mean difference.

* Correspondence author.
E-mail address: s.fairweather-tait@uea.ac.uk (S. Fairweather-Tait).

https://doi.org/10.1016/j.ajcnut.2022.11.007
Received 2 July 2022; Received in revised form 20 October 2022; Accepted 4 November 2022
Available online 19 December 2022

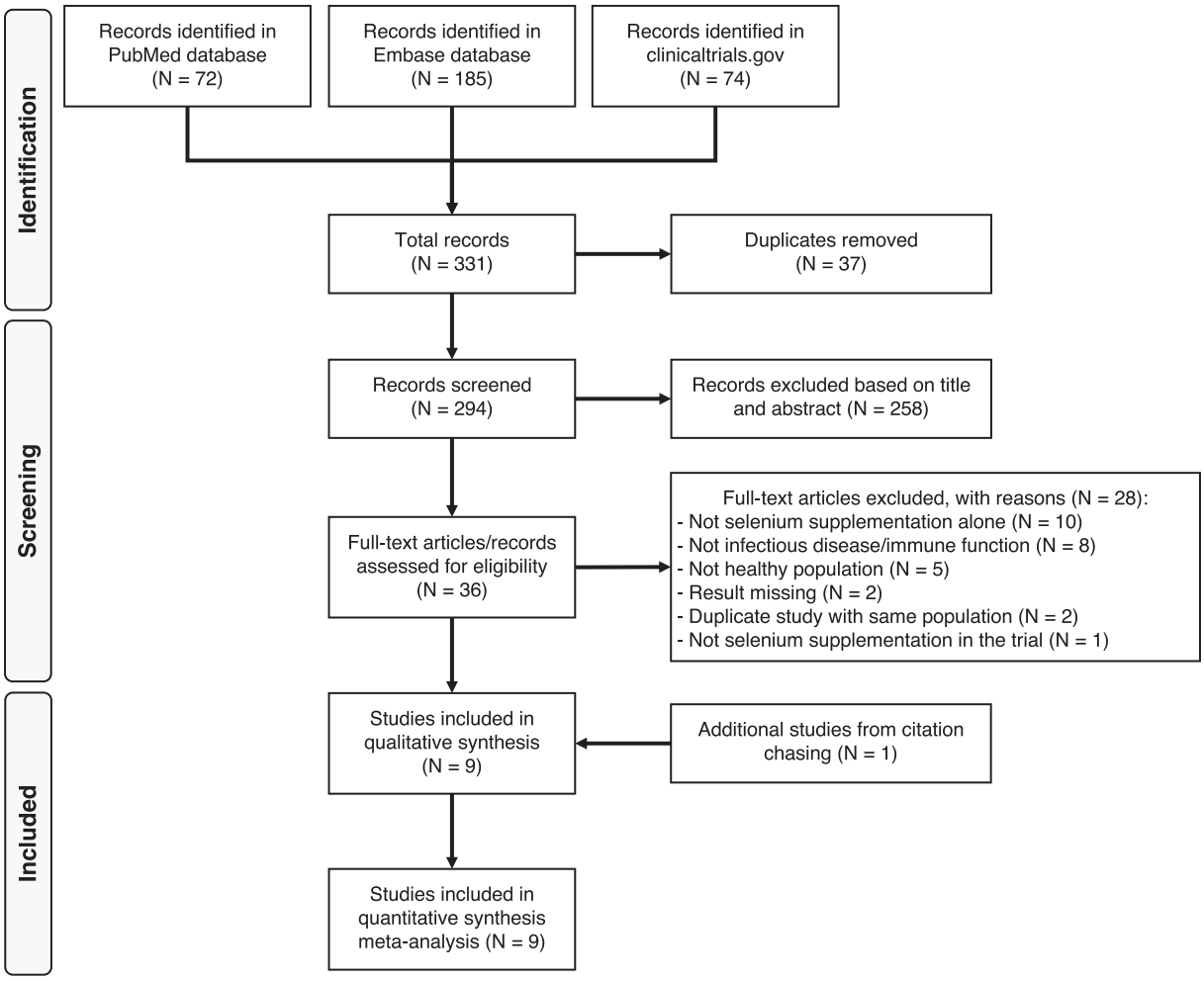

Figure 1. Flow chart for study identification in online databases and clinicaltrials.gov registry.

peroxidases, thioredoxin reductases, iodothyronine deiodinases, methionine-R-sulfoxide reductase B1, and selenophosphate synthetase 2 [9]. For this reason and after some laboratory studies [10], selenium deficiency has been suggested as affecting 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 when it was mainly found in parts of the country where the common feature was low selenium concentrations in soils and foods locally produced [11]. However, some epidemiologic 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]. Coxsackievirus 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 indicate 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 the immune system/function has 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 antipathogen 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. Given 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.

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 healthy men aged 36–50 y, with no history of cardiovascular, pulmonary, and psychiatric diseases, with low-Se status (<70 μg/L)	Yes	DB	11 wk Sep 1981 to Dec 1981	Se–yeast (200 μg/d) + 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 Specific 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 min): Control 810 vs. 1010 PHA 57,600 vs. 57,800 ConA 34,800 vs. 32,100- Phagocytosis of *S. aureus* (CFU/104 granulocytes): 2870 vs. 3220- Number of ingested bacteria viable after 1 h: 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 y not taking medications	Yes[4]	DB	15 wk (1999–2000)	Sodium selenite (50 or 100 μg/d)	22 C 22 T50 22 T100	Mean Baseline: C: 78.96	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

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
		with low-Se status (<94.75 μg/L)						T50: 78.17 T100: 82.12 End of trial: C: 83.5 T50: 91.7 T100: 103.3 (from figure)	and poliovirus antibody production and detection rate Specific response: cytokine levels	with poliovirus antigen derived from the same live attenuated poliovirus vaccine given in vivo was assessed at 0, 7, 14, and 21 d after vaccination.- 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, especially CD8+. Similarly, NK cytotoxicity increased with increasing Se compared with 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 d: 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 groups than in placebo group, indicating more rapid clearance. Also, mutations in the poliovirus were detected with much higher rate in the placebo group compared with Se-treated groups.
Hawkes, 2001 (37)	United States, California	11 healthy men aged 26–45 y with weight for height lower than 125% of ideal and without chronic diseases or medication use	Yes	DB	99 d	Low-Se (13 μg/d) and high-Se diet (297 μg/d) 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 Specific 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. Diphtheria titer for secondary response showed to be higher in high-Se group at the end of the trial. WBCs 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% increased in low-Se. WBC

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
										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 titer 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). Diphtheria: no. at difference at baseline (1500 vs. 14100 low-Se /2100 vs. 15,400 high-Se), but higher levels in high-Se group (14,700 vs. 16,600 Se-low/12,400 vs. 23,600 Se-high).- WBC (baseline vs. final in low- and high-Se diets; thou/mm^3): WBC 4.1 vs. 4.5 low-Se/6.1 vs. 5.8 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/

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
										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 (% lysis) 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- DHT skin response assessed with total diameter and number of indurations at 48 h and 72 h to 7 antigens: tuberculin-purified protein derivative; mumps; tetanus toxoid; candida; trichophyton; streptokinase streptase; coccidioidin. No effect of Se.
Hawkes, 2009 (38)	United States, California	42 healthy nonsmoking men aged 18–45 y with self-reported absence of diseases, clinically normal blood count and blood chemistry, and without obesity	Yes	DB	48 wk	Low-Se vs. High-Se (300 μg/d selenized yeast)	20 C 22 T	Mean ± SD Baseline: Low-Se: 146 ± 19 High-Se: 142 ± 19 End of trial: Low-Se: 141 ± 18	Specific response: WBC levels+ DHT Specific response: NK cell number	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 and NK cells expressing IL2 receptor. DHT skin response decrease by 57% in low-Se diet, whereas decreased ~20%–25% in high-Se diet- WBC levels (cells/μL whole blood):

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
								High-Se: 228 ± 63		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- DHT skin response assessed with total diameter and number of indurations at 48 h and 72 h to 5 antigens: tuberculin-purified protein derivative; mumps; tetanus toxoid; candida; trichophyton). DHT response decrease by 57% in low-Se, whereas decreased ~20%–25% in high-Se. Response to all 5 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).
Ivory, 2017 (28)	United Kingdom, Norfolk	119 (M/F: 54/65) healthy subjects aged 50–64 y with normal hematology, blood chemistry, blood pressure levels and BMI >18.5 and <35 with low-Se status (Se <110 μg/L)	Yes	DB	12 wk	Group SeY: selenized yeast (Se methionine 50, 100, or 200 μg/d) Group SeO: Se-rich onions with 50 Se μg/d	20 C 20 T50 21 T100 23 T200 17 CO 18 TO	Mean ± SD[5] Baseline SeY C: 92.0 (11.9) 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	Specific response: Proliferating T cells after flu vaccination +cytolytic granules Specific response: NK cells + cytokines levels	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 group treated with 100 μg Se/d compared with those treated with both 50 and 200 μg Se/d at week 12. Similarly, cytolytic granules were lower in the group treated with 200 μg Se/d compared with all other SeY groups, whereas in the SeO group, Granzyme B levels were higher in the Se-rich onion group. Cytotoxic cell levels were not affected by Se supplementation.- Proliferating T cells at weeks 10 and

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
								± 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		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/d, but a lower increase occurred in 200 Se μg/d group compared with 100 Se μg/d after flu vaccination at week 12.- Cytolytic granules: Lower granzyme B and perforin in 200 Se μg/d group compared with placebo at either week 10 or 12. Granzyme B levels higher in Se-rich onion group compared with non–Se-rich onion.- Cytotoxic cells: Se supplementation as either SeY or SeO did not have any effects of number of any additional cytotoxic cell subsets 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)	United States, New York University Dental Center	32 (M/F: 27/5) healthy subjects aged 24–36 y divided into 2 subtrials: – CL Group: 21 (M/F: 16/5) subjects assessing cytotoxic lymphocytes – NK group: 11 men, assessing NK cells	Yes[6]	NI	8 wk	Sodium selenite (200 μg/d)	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 C: 122.0 ± 4.0 T: 120.0 ±	Specific response: lymphocytes activity Specific response: NK cell activity	Average BMI of 25 in all groups 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 8 wk resulted in increased lytic activity in Raji tumor cells compared with baseline as well as control group.- CL group: Nonetheless, cytotoxic lytic activity of lymphocytes against Raji tumor cells was higher in the Se-treated group compared with control (45.6% vs. 27.6%).

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
								7.0 End of trial C: 122.0 ± 10.0 T: 114.0 ± 4.0		Despite 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 with placebo)- NK group: Increase in NK lytic activity against Raji tumor cells in Se-treated group compared with control (+108%)
Peretz, 1991 (25)	Belgium, Brussels	22 (M/F: 7/15) elderly institutionalized (≥1 y) subjects aged >65 y with no history of severe disabling diseases (e.g., cancer, cirrhosis, or diabetes)	NI	DB	6 mo	Selenized yeast (100 μg/d)	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	BMI: 28±6 in T, 24±6 in C Lymphocytes proliferation appeared to increase in the Se-treated group after stimulation with 1 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 6 mo), whereas no effects were found in control group as well as 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 with pool serum.
Roy, 1994 (24)	New York University Dental Center	22 (M/F: 12/10) healthy people age 24–36 y	Yes[6]	NI	8 wk	Sodium selenite (200 μg/d)	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 48 h but 19.1% after 72 h.
Wood, 2000 (26)	United States, Southern Arizona	21 (M/F: 13/9) healthy nonsmoking people aged 57–84 y with no history of chronic diseases (e.g.,	Yes	SB	6 mo	Selenized yeast (400 μg/d)	8 C 8 T	Mean ± SD Baseline: C: 155 ±	Specific response: Total T cells and subgroups Specific response:	Se supplementation did not affect total WBC levels, whereas increased T cells and particularly T helper CD4+. Conversely, NK cells showed

(*continued on next page*)

Table 1 (*continued*)

Reference	Country	Population[1]	R	Blinding	Duration[2]	Intervention	Groups	Plasma Se levels (μg/L)	Outcomes	Results
		cancer, cardiovascular diseases, or diabetes). Analyzed 16 (8C + 8T due to 22% attrition rate)						6.0 T: 129 ± 4.8 End of trial C: 153.8 T: 141.3	Total WBC and NK cells and activity	similar levels in Se-treated group but NK activity resulted higher.- WBC counts: No changes in WBC differentials due to supplementation. Total B cells did not change in any group. Total T cells increased >50% in Se-treated group, whereas 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

BMI: body mass index measured as weight (in kg)/height (in m^2); 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;

[1] Male/female ratio reported whenever possible;

[2] Duration in: days (d), weeks (wk) or months (mo);

[3] Data reported in Levander et al., 1983 (57);

[4] From personal communication with authors;

[5] Data reported in previous report Hurst et al., 2010 (58);

[6] Included gender, race, age, body weight, height, dietary habits, and history of vitamin intake, tobacco, and alcohol in the randomization process.

Methods

After registering the protocol in PROSPERO (registration no. CRD42022312280), we carried out a systematic review according to the PRISMA guidelines [21].

Study identification and selection

We conducted a search of online literature databases (PubMed/MEDLINE and EMBASE) and clinicaltrials.gov up to 17 October, 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 Supplemental Table 1. Inclusion criteria were as follows: being an epidemiologic study in healthy humans, selenium being the only difference between experimental and control groups, 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 assessed the risk of bias of the included studies using the RoB 2.0 tool of the Cochrane Collaboration [22] using the subsequent 5 domains: *1)* bias arising from the 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; and *5)* bias in selection of the reported results. For each domain the judgment can be low risk, some concerns, or high risk. Overall, we judged a study at "low risk" of bias if it had low risk in all domains, whereas we judged it as having "some concerns" when at least 1 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 1 domain.

Data extraction

We extracted the following data from included reports: first author name, publication year, country, type, and 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 the difference between intervention and control group at the end of the intervention; and outcomes of interests related to infectious disease and immune function. When data were mentioned but not explicitly reported or only partially available, for example, 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 SD, SE, or IQR. When data were available only from figures, we sought to extract mean levels along with SD or SE from figures. About 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 2 studies because they were no longer working [23,24]. For 1 study, we did not get any answer despite the availability of email addresses from recent articles [25] and for another study, the corresponding author confirmed that original data

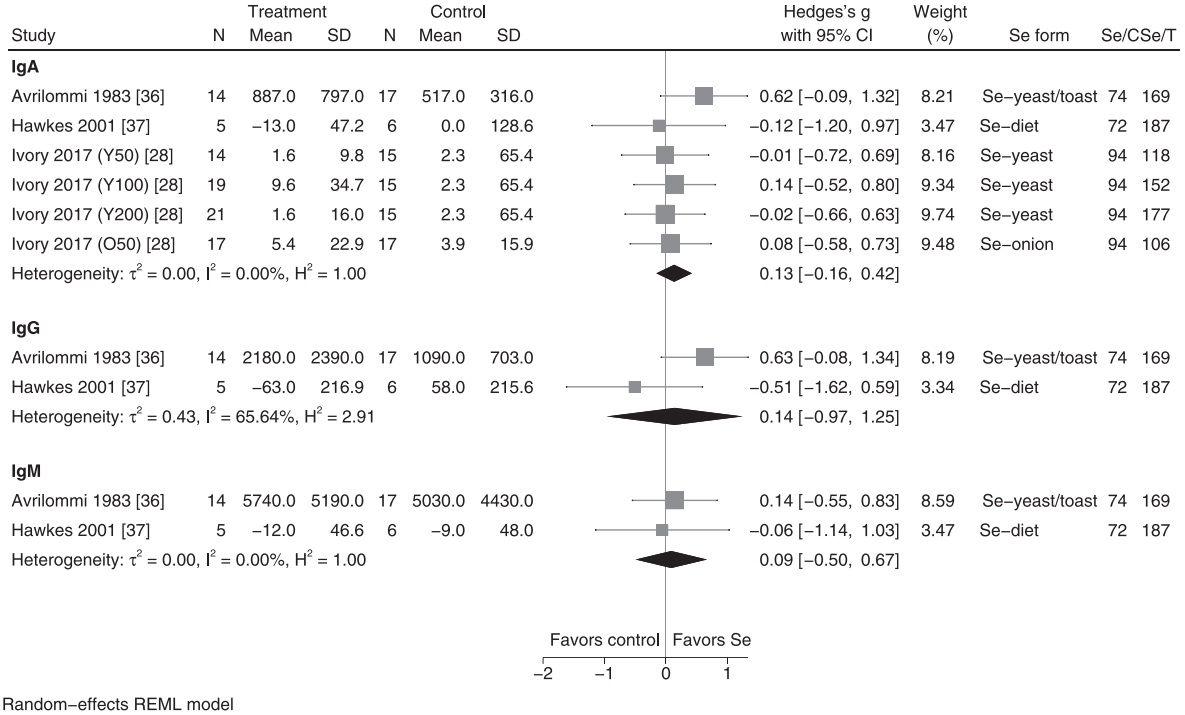

Figure 2. Forest plot of Hedge's g SMDs for Ig levels, all studies. The area of each gray square is proportional to the inverse of the variance of the estimated SMD, and horizontal lines represent the 95% CI. Black diamonds represent point estimates of overall SMD for each group. The solid vertical line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

were no longer available [26]. Finally, for 1 study, information was available about study design (e.g., randomization, blinding), but raw data were no longer available [27], but we were able to retrieve original data from a later study for use in the meta-analysis [28]. To perform quantitative analysis, in 5 out of 9 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 2 studies for that endpoint were available. When 3 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 the higher versus the lowest category, that is, treated versus the control group. We did this through computation of Hedge's g standardized mean differences (SMD) along with their 95% CI, due to heterogeneity in units of measurements of outcomes when at least 2 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 1-stage methodology [29, 30], an approach for implementation of dose–response meta-analysis based on a weighted mixed effects model and using cubic splines, which enables the pooling of results from all studies when at least 2 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, as a reference dose, we used the median value of the set of studies alternatively considered in each specific analysis. All analyses were carried out using "meta" and "drmeta" routines of Stata statistical software (Stata 17.0-SE 2021, StataCorp LLC). For all data analyses we did not use null hypothesis testing and *P* value cutpoints, following 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 the 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 9 studies in the final analysis [23–28, 36–38], 1 of which was retrieved through citation chasing [37]. Reasons for exclusion after full-text evaluation are reported in detail in Supplemental Table 2.

Overall, the characteristics of the 9 included studies are reported in Table 1. Five studies were carried out in North America/United States [23,24,26,37,38], 2 in the United Kingdom [27,28], 1 in Belgium [25], and 1 in Finland [36]. Overall, they included 370 participants (220 in selenium-treatment groups and 150 in the control groups) with ages ranging from 18 to 64 years in all but 2 studies; 1 was undertaken in elderly institutionalized subjects aged ≥65 years [25] and 1 in subjects aged 57–84 years [26]. Three studies recruited only male participants [36–38), whereas other 6 studies recruited both male and female participants, although none reporting gender-stratified analysis. The duration of the trials ranged from 8 to 48 weeks. Selenium supplementation occurred in the inorganic form using sodium selenite in 3 trials [23,24,27]. The intervention doses varied with 50 and 100 μg/d used in 1 trial [27] and 200 μg/d in the remaining 2 trials [23,24]. Conversely, organic selenium was administered in the form of selenized yeast in 5 trials [25,26,28,36,38]. Doses varied from 50 up to 400 μg/d, with some trials having 2 or more intervention groups at increasing doses of selenium. In addition, 2 trials also used Se-rich foods to increase selenium intake in the intervention groups. Specifically, 1 trial administered wheat toast made with Se-rich flour [36] and other Se-enriched onions [28]. Finally, Se-low and Se-high diets were administered to study participants in 1 trial in the control and intervention groups directly providing 3 daily meals made with foods, namely, rice and beef staples, from different geographic 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

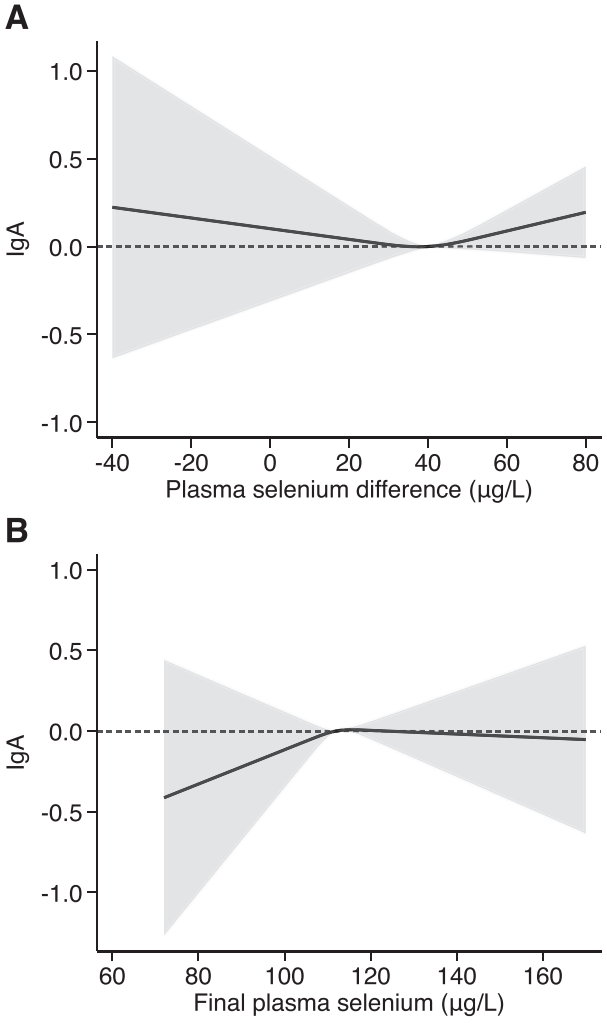

Figure 3. Dose–response meta-analysis of changes of IgA levels according to plasma selenium difference (A and C) and final levels (B and D) of plasma selenium (μg/L) between selenium-supplemented and control groups at the end of the trials, all studies, $N = 3$ [28,36,37]. Solid black line represents 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 3 knots at fixed cutpoints (10th, 50th and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The gray area represents 95% CI. The short-dashed line represents the null effect, SMD = 0. SMD, standardized mean difference.

between 70 and 118 μg/L (mean, 110 μg/L) and between 66 and 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 2 trials, plasma concentrations of selenium did not increase in the intervention groups and were substantially similar to those of the control groups [23,24]. Finally, in 1 trial [26], selenium supplementation did increase the baseline selenium concentrations (from 129 to 142 μg/L), whereas the control group showed constantly higher concentrations at both baseline and the end of the trial (155 vs. 154 μg/L).

Results of the risk of bias assessment are reported in Supplemental 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-

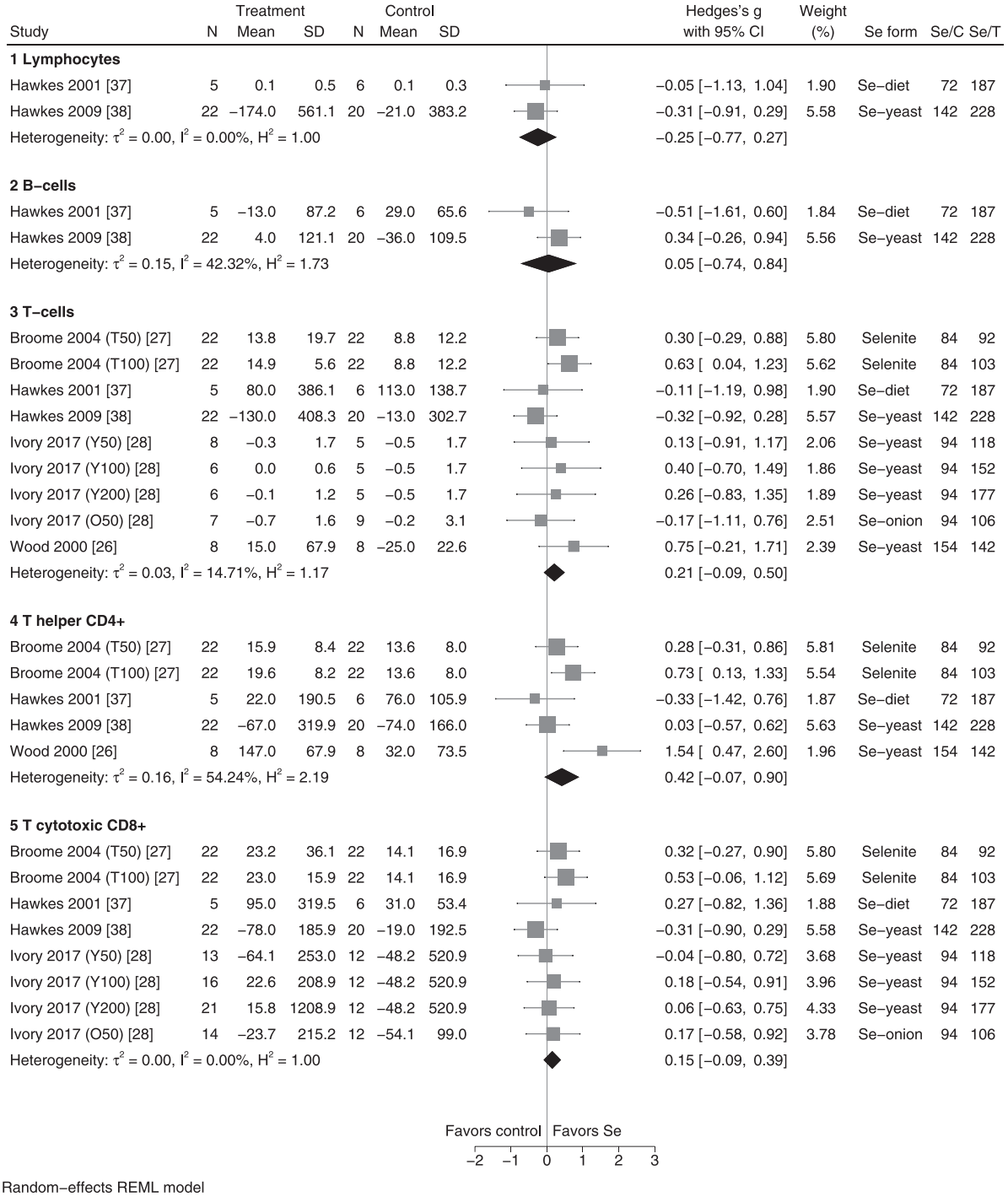

Figure 4. Forest plot of Hedge's g SMDs for lymphocyte levels, all studies. The area of each gray square is proportional to the inverse of the variance of the estimated SMD, and horizontal lines represent the 95% CI. Black diamonds represent point estimates of overall SMD for each group. The solid vertical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

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 2 trials were judged as having a "high risk" of bias due to deviations from the intended interventions because no information about blinding was 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 the 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, that is, 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 the 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 to 110 μg/L, although 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 2 studies, selenium supplementation seems to be associated with a decrease in total lymphocyte levels (SMD = −0.25; 95% CI: −0.77, 0.27), whereas it had no effect on B cells. Conversely, a slight increase in T cells was noted, although it is very imprecise and when only studies at low risk of bias were considered, the increase was less (Supplemental Figure 1).

Figure 5 presents the dose–response analysis for T cells and T CD8+ cells for which a sufficient number of studies were available. When considering both difference and final selenium concentrations, an

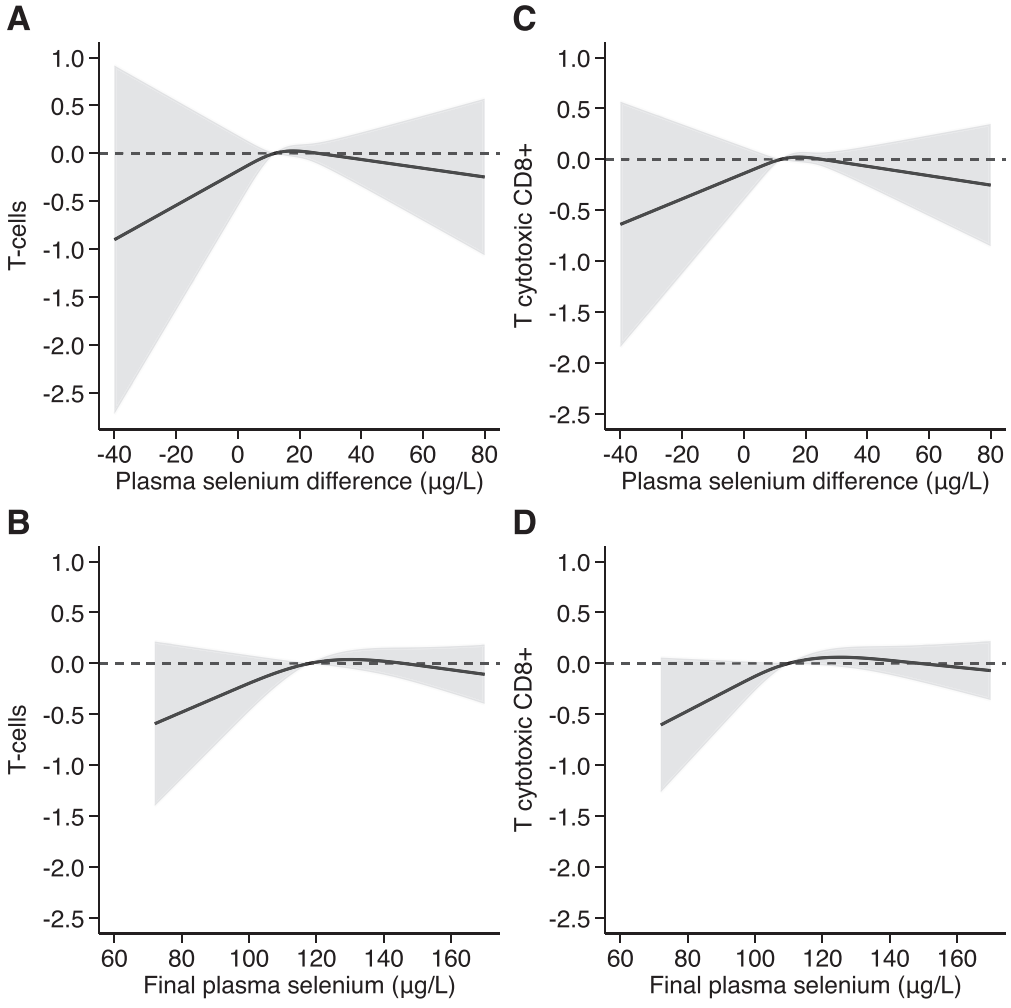

Figure 5. Dose–response meta-analysis of changes in T cells and T-cytotoxic CD8+ levels according to plasma selenium difference (A and C) and final levels (B and D) of plasma selenium (μg/L) between selenium-supplemented and control groups at the end of the trials, all studies, $N = 5$ in (A and B) [26–28,37,38], $N = 4$ 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 3 knots at fixed cutpoints (10th, 50th, and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The gray area represents 95% CI. The short-dashed line represents the null effect, SMD = 0.

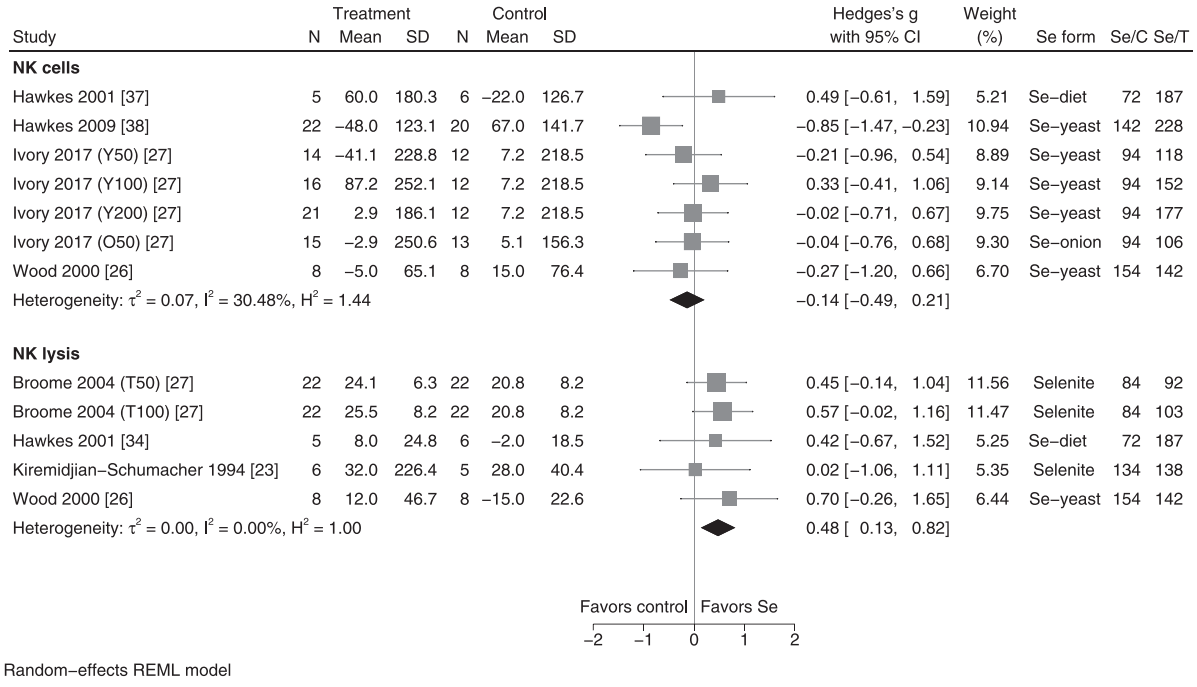

Figure 6. Forest plot of Hedge's g SMDs for NK cells and lysis, all studies. The area of each gray square is proportional to the inverse of the variance of the estimated SMD, and horizontal lines represent the 95% CI. Black diamonds represent point estimates of overall SMD for each group. The solid vertical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

increase can be noted until approximately 10 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 (Supplemental Figure 2).

Figure 6 presents the SMD for 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 (Supplemental Figure 3). In the dose–response analysis for NK cells, increases in plasma selenium seem to be associated with an increase in NK cells until 140 μg/L but with further increase an inverse relationship 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 (Supplemental Figure 4).

Supplemental Figure 5 presents SMD for expression or IL-2 receptors reported in 2 studies indicating no effect of selenium supplementation (either in the form of organic or inorganic selenium), with the same results after exclusion of 1 study at high risk of bias (Supplemental Figure 6). Supplemental Figure 7 shows the effect of selenium supplementation on cytokine levels, namely, IL-10 and IFN-gamma from 2 studies, both at low risk of bias, suggesting a slight though imprecise increase (IL-10: SMD = 0.07, 95% CI: −0.18, 0.33; IFN-gamma: SMD = 0.20, 95% CI: −0.05, 0.45). Supplemental Figure 8 shows results of lymphocyte proliferation in either absence or presence of external stimuli (e.g., different mitogens or vaccination) as assessed through total lymphocyte proliferation in all but 1 study assessing T-cell proliferation [28]. All studies are at low risk of bias. Overall, lymphocyte proliferation seems to be unaffected by selenium supplementation. For the analysis including IL-2 receptor, cytokine levels, and lymphocyte proliferation, we could not perform dose–response meta-analysis due to the limited number of studies.

Discussion

This review, to the best of our knowledge, the first to meta-analyze human trials on selenium supplementation and immunologic endpoints using a dose–response approach, provides little evidence of the beneficial effects of selenium supplementation on immune function as assessed through the evaluation of levels of immunoglobulins, and white blood cells, particularly lymphocytes and NK cells. In particular, the dose–response meta-analysis indicates that an increase in plasma selenium concentration above 100 μg/L does not further increase IgA levels nor T cells, as seen at lower levels, suggesting the occurrence of a possible beneficial effect that corresponds to an intake of 70 μg/d, namely, the Dietary Reference Value (DRV) for selenium [5], using a conversion factor of 1.5 as previously suggested [39]. The pattern of association becomes more complex regarding NK cell count, for which an inverted U-shaped relation emerged, with lower numbers of these cells both below and above 120 μg/L (80 μg/d). Such a U-shaped pattern is not unusual for a nutrient, considering that both levels of too-low and too-high exposure may induce adverse effects. The only beneficial effect of selenium emerged for NK lysis, but data did not allow us to test any dose–response relation or the possible presence of a U-shaped curve. The dose–response curve indicated that increased selenium intake at the lowest range of the selenium intake tested in the trials was beneficial, but that achieving intakes above the DRV of ~70 μg/d, as implied by the 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 of IgG against SARS-CoV-2 in subjects with different dietary intakes of selenium or habitual selenium supplementation found no effect of the selenium status on this parameter, which is consistent with the results of the trials [40].

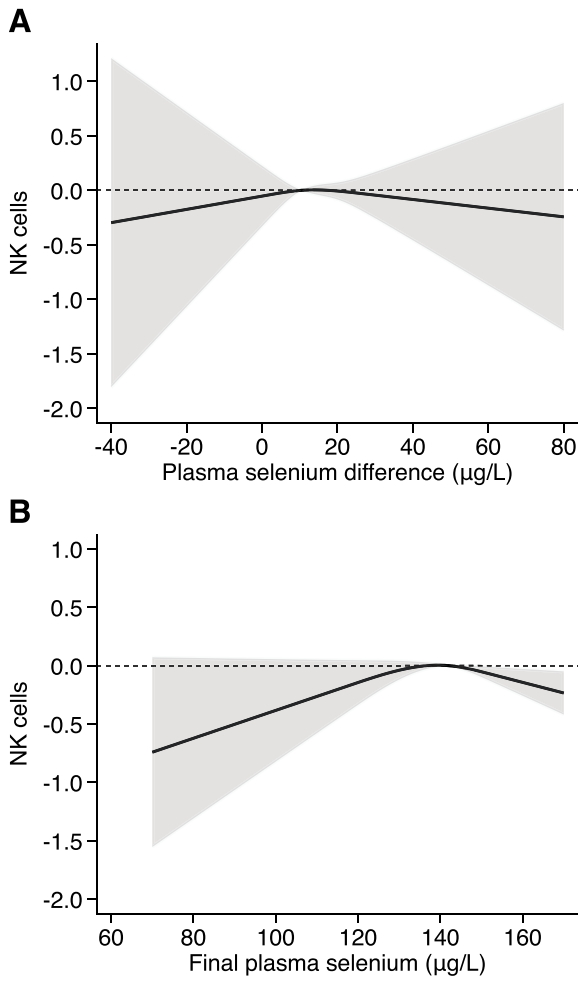

Figure 7. Dose–response meta-analysis of changes in NK cell levels according to difference (A) and final (B) levels of plasma selenium (µg/mL) between selenium-supplemented and control groups at the end of the trials, all studies, $N = 4$ [26,28,37,38]. Solid black line represents the effect with variation of SMD (y-axis) according to the plasma selenium levels (x-axis). The curves are designed using restricted cubic spline method using 3 knots at fixed cutpoints (10th, 50th, and 90th percentiles) and considering the median value (50th) of such distribution as reference point. The gray area represents 95% CI. The short-dashed line represents the null effect, SMD = 0. SMD, standardized mean difference.

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, although such a relationship could not be investigated through a dose–response approach because 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 humans is 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 ~86–93 µg/d [1, 39]. Conversely, selenium levels in the European populations are somewhat lower, ranging from 50 to 120 µg/L [5,47,48]. For these reasons and according to our findings, the intake of selenium in these populations can be considered adequate, with no need to increase selenium through supplementation concerning improving immune function. About the presence of a U-shaped 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/d 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 ~100 µg/L, whereas higher levels may be associated with 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 lacking as no trials have been performed so far [52], and some evidence from 2 recent Mendelian randomization studies investigating this issue was 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 a 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. First, 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) even precluded such analysis. For some of the included studies, we extracted numerical data from figures to perform quantitative analysis whenever possible, thus possibly inducing some additional amount of imprecision of the individual estimates. Second, 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. Concerning selection bias, most of the studies excluded participants with chronic diseases, especially cancer, cardiovascular diseases, or diabetes, and some also included cutoff levels of BMI 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 2 trials included subjects younger than 65 y, and those including older

participants found consistent results with 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 concerning immunologic 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 the immune system.

Overall, the assessment and the meta-analysis of the experimental human studies investigating the immunologic effects of selenium administration yielded limited evidence of the 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 ~70 μg/d, whereas higher intakes were associated with null or even adverse effects.

We are grateful to Jack Dainty, Malcolm Jackson, and Claudio Nicoletti for their valuable suggestions and providing data for the meta-analysis. The authors' responsibilities were as follows—SF-T: conceived the study with MV and TF; TF: extracted data with the supervision of SF-T and MV, performed the statistical analysis, interpreted the data with SF-T and MV, and prepared the first draft manuscript with substantial contribution of other authors; and all authors: retrieved and selected relevant articles and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending an application and the approval of the corresponding author.

Funding

The authors reported no funding received for this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2022.11.007.

References

[1] M. Vinceti, T. Filippini, L.A. Wise, Environmental selenium and human health: an update, Curr Environ Health Rep 5 (4) (2018) 464–485.
[2] S.J. Fairweather-Tait, Y. Bao, M.R. Broadley, R. Collings, D. Ford, J.E. Hesketh, et al., Selenium in human health and disease, Antioxid Redox Signal 14 (7) (2011) 1337–1383.
[3] V.M. Labunskyy, D.L. Hatfield, V.N. Gladyshev, Selenoproteins: molecular pathways and physiological roles, Physiol Rev 94 (3) (2014) 739–777.
[4] M. Vinceti, T. Filippini, E. Jablonska, Y. Saito, L.A. Wise, Safety of selenium exposure and limitations of selenoprotein maximization: molecular and epidemiologic perspectives, Environ Res 211 (2022), 113092.
[5] EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), Scientific opinion on dietary reference values for selenium, EFSA J 12 (10) (2014) 3846.
[6] Institute of Medicine, Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids, National Academies Press, Washington (DC), 2000.
[7] World Health Organization, Vitamin and mineral requirements in human nutrition [Internet], 2nd edn., World Health Organization, Geneva, Switzerland, 2004. Date accessed), https://apps.who.int/iris/handle/10665/42716. (Accessed 17 October 2022).
[8] A.P. Kipp, D. Strohm, R. Brigelius-Flohe, L. Schomburg, A. Bechthold, E. Leschik-Bonnet, et al., German Nutrition Society. Revised reference values for selenium intake, J Trace Elem Med Biol 32 (2015) 195–199.
[9] J.C. Avery, P.R. Hoffmann, Selenium, selenoproteins, and immunity, Nutrients 10 (9) (2018) 1203.
[10] M.A. Beck, Selenium and vitamin E status: impact on viral pathogenicity, J Nutr 137 (5) (2007) 1338–1340.
[11] C. Yan, R. Luo, F. Li, M. Liu, J. Li, W. Hua, et al., The epidemiological status, environmental and genetic factors in the etiology of Keshan disease, Cardiovasc Endocrinol Metab 10 (1) (2021) 14–21.
[12] L.Q. Ren, X.J. Li, G.S. Li, Z.T. Zhao, B. Sun, F. Sun, Coxsackievirus B3 infection and its mutation in Keshan disease, World J Gastroenterol 10 (22) (2004) 3299–3302.
[13] M.A. Beck, P.C. Kolbeck, Q. Shi, L.H. Rohr, V.C. Morris, O.A. Levander, Increased virulence of a human enterovirus (Coxsackievirus B3) in selenium-deficient mice, J Infect Dis 170 (2) (1994) 351–357.
[14] C. Cermelli, M. Vinceti, E. Scaltriti, E. Bazzani, F. Beretti, G. Vivoli, et al., Selenite inhibition of Coxsackie virus B5 replication: implications on the etiology of Keshan disease, J Trace Elem Med Biol 16 (1) (2002) 41–46.
[15] Y. Molin, P. Frisk, N.G. Ilback, Viral RNA kinetics is associated with changes in trace elements in target organs of Coxsackie virus B3 infection, Microbes Infect 11 (4) (2009) 493–499.
[16] O.A. Levander, M.A. Beck, Interacting nutritional and infectious etiologies of Keshan disease. Insights from coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E, Biol Trace Elem Res 56 (1) (1997) 5–21.
[17] A.D. Smith, S. Botero, O.A. Levander, Copper deficiency increases the virulence of amyocarditic and myocarditic strains of coxsackievirus B3 in mice, J Nutr 138 (5) (2008) 849–855.
[18] H. Gill, G. Walker, Selenium, immune function and resistance to viral infections, Nutr Diet 65 (Suppl. 3) (2008) S41–S47.
[19] Z. Huang, A.H. Rose, P.R. Hoffmann, The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities, Antioxid Redox Signal 16 (7) (2012) 705–743.
[20] Scientific Advisory Committee on Nutrition, SACN rapid scoping exercise: nutrition, immunity and COVID-19 [Internet], Office for Health Improvement and Disparities, UK, 2020. Date accessed), https://www.gov.uk/government/publications/sacn-rapid-scoping-exercise-nutrition-immunity-and-covid-19. (Accessed 17 October 2022).
[21] M.J. Page, J.E. McKenzie, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, et al., The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, J Clin Epidemiol 134 (2021) 178–189.
[22] J.A.C. Sterne, J. Savovic, M.J. Page, R.G. Elbers, N.S. Blencowe, I. Boutron, et al., RoB 2: a revised tool for assessing risk of bias in randomised trials, BMJ 366 (2019) l4898.
[23] L. Kiremidjian-Schumacher, M. Roy, H.I. Wishe, M.W. Cohen, G. Stotzky, Supplementation with selenium and human immune cell functions. II. Effect on cytotoxic lymphocytes and natural killer cells, Biol Trace Elem Res 41 (1–2) (1994) 115–127.
[24] M. Roy, L. Kiremidjian-Schumacher, H.I. Wishe, M.W. Cohen, G. Stotzky, Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression, Biol Trace Elem Res 41 (1–2) (1994) 103–114.
[25] A. Peretz, J. Nève, J. Desmedt, J. Duchateau, M. Dramaix, J.P. Famaey, Lymphocyte response is enhanced by supplementation of elderly subjects with selenium-enriched yeast, Am J Clin Nutr 53 (5) (1991) 1323–1328.
[26] S.M. Wood, C. Beckham, A. Yosioka, H. Darban, R.R. Watson, β-Carotene and selenium supplementation enhances immune response in aged humans, Integr Med 2 (2–3) (2000) 85–92.
[27] C.S. Broome, F. McArdle, J.A. Kyle, F. Andrews, N.M. Lowe, C.A. Hart, et al., An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status, Am J Clin Nutr 80 (1) (2004) 154–162.
[28] K. Ivory, E. Prieto, C. Spinks, C.N. Armah, A.J. Goldson, J.R. Dainty, et al., Selenium supplementation has beneficial and detrimental effects on immunity to influenza vaccine in older adults, Clin Nutr 36 (2) (2017) 407–415.
[29] A. Crippa, A. Discacciati, M. Bottai, D. Spiegelman, N. Orsini, One-stage dose-response meta-analysis for aggregated data, Stat Methods Med Res 28 (5) (2019) 1579–1596.
[30] N. Orsini, S.C. Larsson, G. Salanti, Systematic reviews in health research, meta-analysis in context, 3rd edn., John Wiley & Sons Ltd, Oxford, UK, 2022, pp. 258–269. Chapter 14, Dose–response meta-analysis.
[31] T. Filippini, M. Malavolti, P.K. Whelton, A. Naska, N. Orsini, M. Vinceti, Blood pressure effects of sodium reduction: dose-response meta-analysis of experimental studies, Circulation 143 (16) (2021) 1542–1567.

[32] M. Vinceti, T. Filippini, L.A. Wise, K.J. Rothman, A systematic review and dose-response meta-analysis of exposure to environmental selenium and the risk of type 2 diabetes in nonexperimental studies, Environ Res 197 (2021), 111210.
[33] R.L. Wasserstein, N.A. Lazar, The ASA statement on *p*-values: context, process, and purpose, Am Statist 70 (2) (2016) 129–133.
[34] N. Berselli, T. Filippini, G. Adani, M. Vinceti, Toxicological risk assessment and multi-system health impacts from exposure, Academic Press, London, UK, 2021, pp. 309–321. Chapter 27, Dismissing the use of *P*-values and statistical significance testing in scientific research: new methodological perspectives in toxicology and risk assessment.
[35] S. Greenland, S.J. Senn, K.J. Rothman, J.B. Carlin, C. Poole, S.N. Goodman, et al., Statistical tests, *P* values, confidence intervals, and power: a guide to misinterpretations, Eur J Epidemiol 31 (4) (2016) 337–350.
[36] H. Arvilommi, K. Poikonen, I. Jokinen, O. Muukkonen, L. Räsänen, J. Foreman, et al., Selenium and immune functions in humans, Infect Immun 41 (1) (1983) 185–189.
[37] W.C. Hawkes, D.S. Kelley, P.C. Taylor, The effects of dietary selenium on the immune system in healthy men, Biol Trace Elem Res 81 (3) (2001) 189–213.
[38] W.C. Hawkes, A. Hwang, Z. Alkan, The effect of selenium supplementation on DTH skin responses in healthy North American men, J Trace Elem Med Biol 23 (4) (2009) 272–280.
[39] M. Haldimann, T.Y. Venner, B. Zimmerli, Determination of selenium in the serum of healthy Swiss adults and correlation to dietary intake, J Trace Elem Med Biol 10 (1) (1996) 31–45.
[40] K. Demircan, T.S. Chillon, Q. Sun, R.A. Heller, G.J. Klingenberg, I.M. Hirschbil-Bremer, et al., Humoral immune response to COVID-19 mRNA vaccination in relation to selenium status, Redox Biol 50 (2022), 102242.
[41] C. Ma, P.R. Hoffmann, Selenoproteins as regulators of T cell proliferation, differentiation, and metabolism, Semin Cell Dev Biol 115 (2021) 54–61.
[42] S.J. Fairweather-Tait, T. Filippini, M. Vinceti, Selenium status and immunity, Proc Nutr Soc (2022) 1–7.
[43] C.A. Stone, K. Kawai, R. Kupka, W.W. Fawzi, Role of selenium in HIV infection, Nutr Rev 68 (11) (2010) 671–681.
[44] M. Laclaustra, S. Stranges, A. Navas-Acien, J.M. Ordovas, E. Guallar, Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004, Atherosclerosis 210 (2) (2010) 643–648.
[45] X. Wang, Y.A. Seo, S.K. Park, Serum selenium and non-alcoholic fatty liver disease (NAFLD) in U.S. adults: National Health and Nutrition Examination Survey (NHANES) 2011–2016, Environ Res 197 (2021), 111190.
[46] S. Moon, H.S. Chung, J.M. Yu, H.J. Yoo, J.H. Park, D.S. Kim, et al., Association between serum selenium level and the prevalence of diabetes mellitus in U.S. population, J Trace Elem Med Biol 52 (2019) 83–88.
[47] M. Vinceti, P. Grill, C. Malagoli, T. Filippini, S. Storani, M. Malavolti, et al., Selenium speciation in human serum and its implications for epidemiologic research: a cross-sectional study, J Trace Elem Med Biol 31 (2015) 1–10.
[48] T. Urbano, T. Filippini, D. Lasagni, T. De Luca, P. Grill, S. Sucato, et al., Association of urinary and dietary selenium and of serum selenium species with serum alanine aminotransferase in a healthy Italian population, Antioxidants (Basel) 10 (10) (2021) 1516.
[49] A.G. Renwick, R. Walker, Risk assessment of micronutrients, Toxicol Lett 180 (2) (2008) 123–130.
[50] M. Vinceti, T. Filippini, K.J. Rothman, Selenium exposure and the risk of type 2 diabetes: a systematic review and meta-analysis, Eur J Epidemiol 33 (9) (2018) 789–810.
[51] J.L. Domingo, M. Marques, The effects of some essential and toxic metals/metalloids in COVID-19: a review, Food Chem Toxicol 152 (2021), 112161.
[52] E. Balboni, F. Zagnoli, T. Filippini, S.J. Fairweather-Tait, M. Vinceti, Zinc and selenium supplementation in COVID-19 prevention and treatment: a systematic review of the experimental studies, J Trace Elem Med Biol 71 (2022), 126956.
[53] N. Daniel, E. Bouras, K.K. Tsilidis, D.J. Hughes, Genetically predicted circulating concentrations of micronutrients and COVID-19 susceptibility and severity: a Mendelian randomization study, Front Nutr 9 (2022), 842315.
[54] M.K. Sobczyk, T.R. Gaunt, The effect of circulating zinc, selenium, copper and vitamin K1 on COVID-19 outcomes: a Mendelian randomization study, Nutrients 14 (2) (2022) 233.
[55] O.M. Guillin, C. Vindry, T. Ohlmann, L. Chavatte, Interplay between selenium, selenoproteins and HIV-1 replication in human CD4 T-lymphocytes, Int J Mol Sci 23 (3) (2022) 1394.
[56] B.A. Muzembo, N.R. Ngatu, K. Januka, H.L. Huang, C. Nattadech, T. Suzuki, et al., Selenium supplementation in HIV-infected individuals: a systematic review of randomized controlled trials, Clin Nutr ESPEN 34 (2019) 1–7.
[57] O.A. Levander, G. Alfthan, H. Arvilommi, C.G. Gref, J.K. Huttunen, M. Kataja, et al., Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters, Am J Clin Nutr 37 (6) (1983) 887–897.
[58] R. Hurst, C.N. Armah, J.R. Dainty, D.J. Hart, B. Teucher, A.J. Goldson, et al., Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial, Am J Clin Nutr 91 (4) (2010) 923–931.