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# Supplementary file 2. Risk of Bias and GRADE assessments

Note: Reference numbers refer to the reference list in the review

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# Risk of Bias Summary

## Randomized control trials included in the meta-analysis

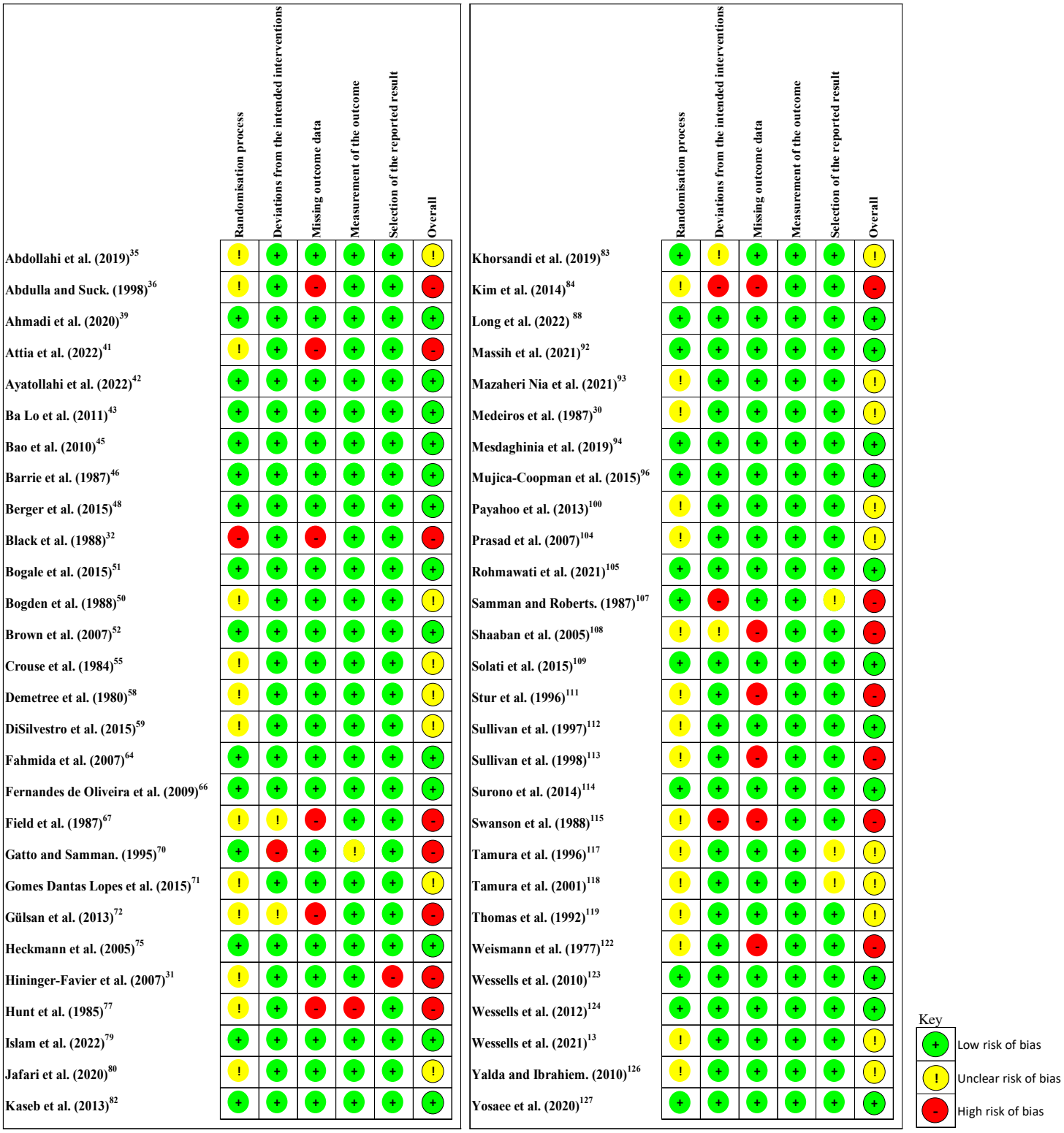


Figure 1. Risk of Bias summary of all randomized control trials included in the meta-analysis, shown as the authors judgment for each RoB2 category for each study included.

## Non-Randomized studies included in the meta-analysis

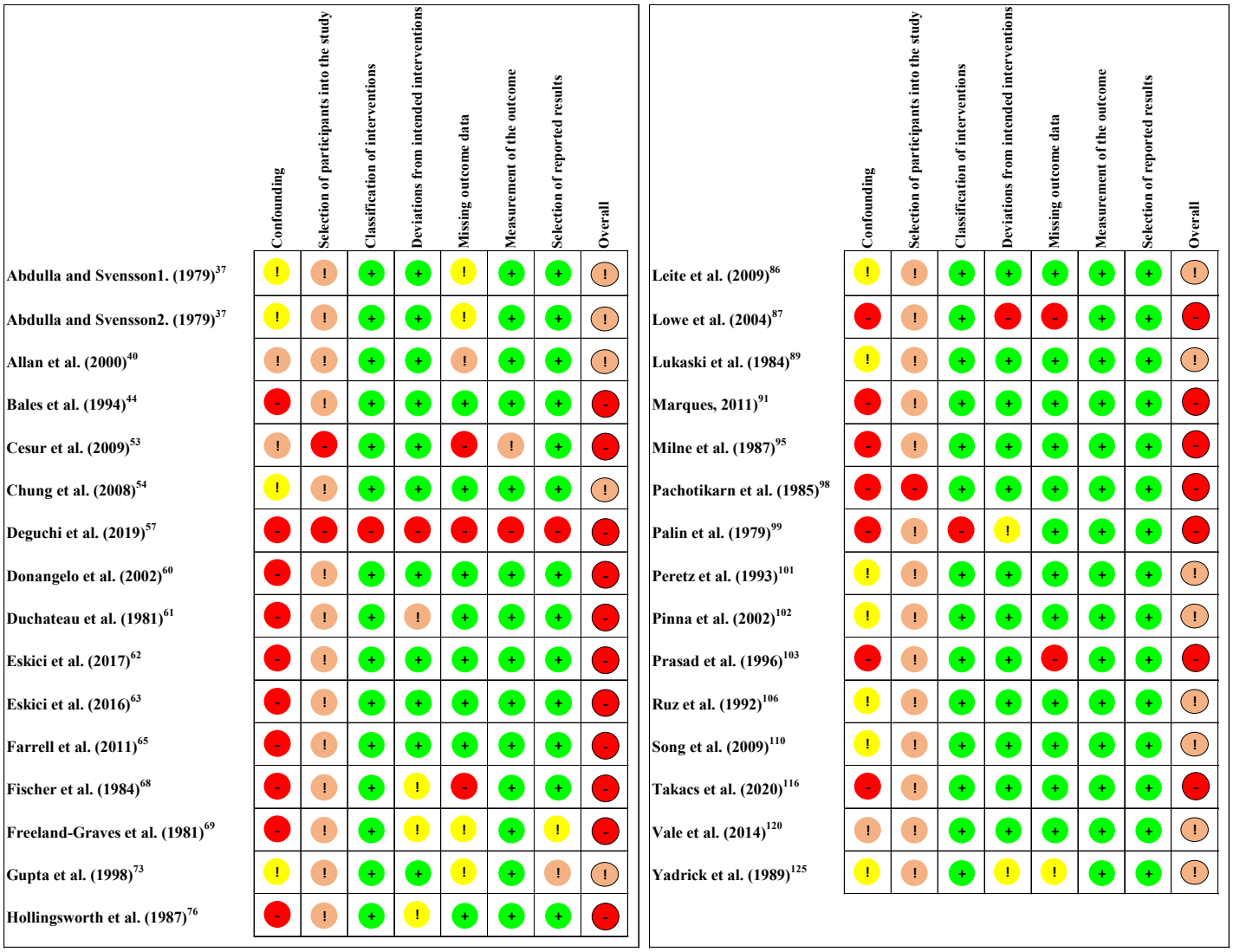


Figure 2. Risk of Bias summary of all non-randomized studies included in the meta-analysis, shown as the authors judgment for each ROBINS-I (Risk of bias in non-randomized studies of interventions) category for each study included.

## Randomized control trials included in the narrative analysis

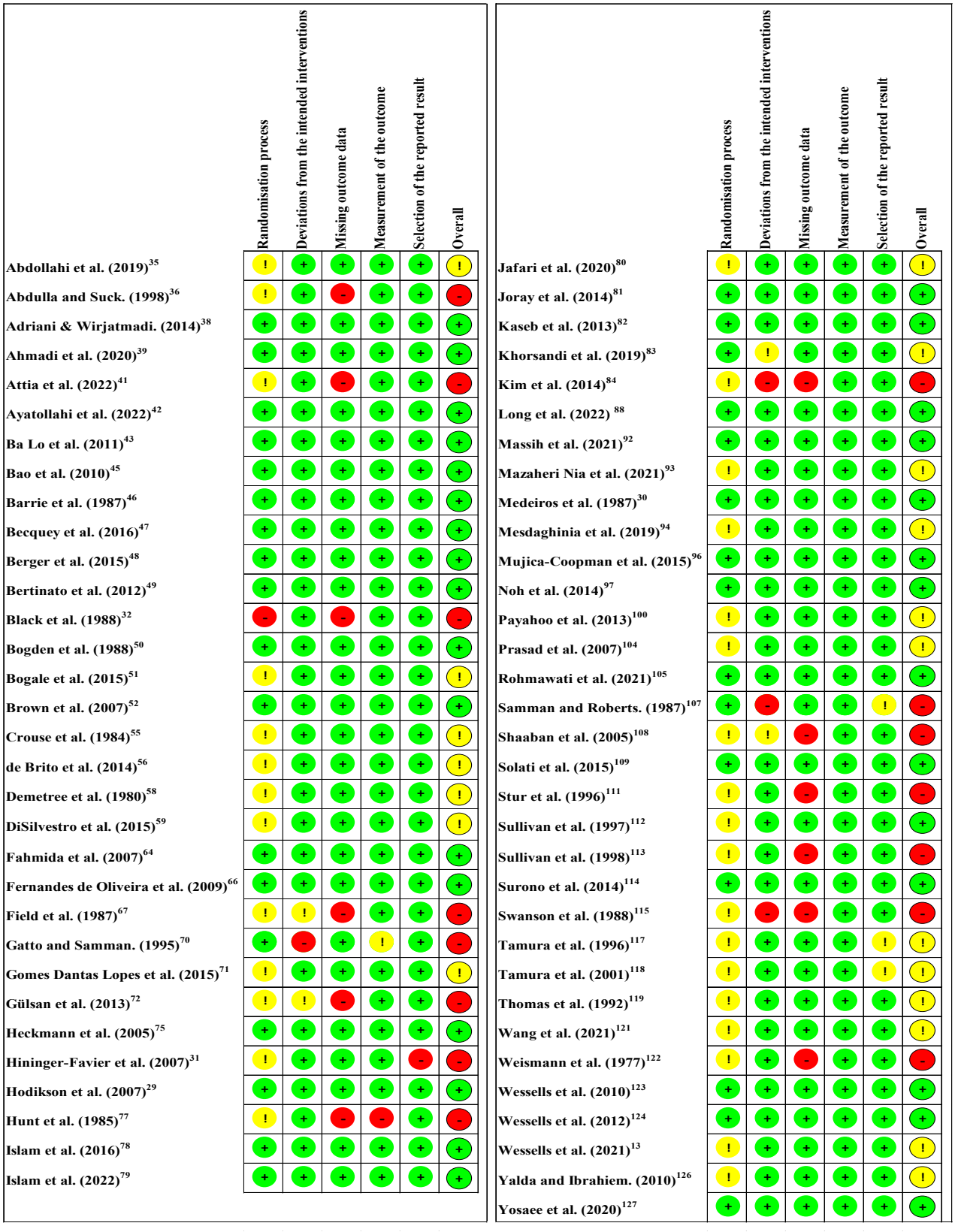


Figure 3. Risk of Bias summary of all randomized control trials included in the narrative analysis, shown as the authors judgment for each RoB2 category for each study included.

## Non-Randomized studies included in the narrative analysis

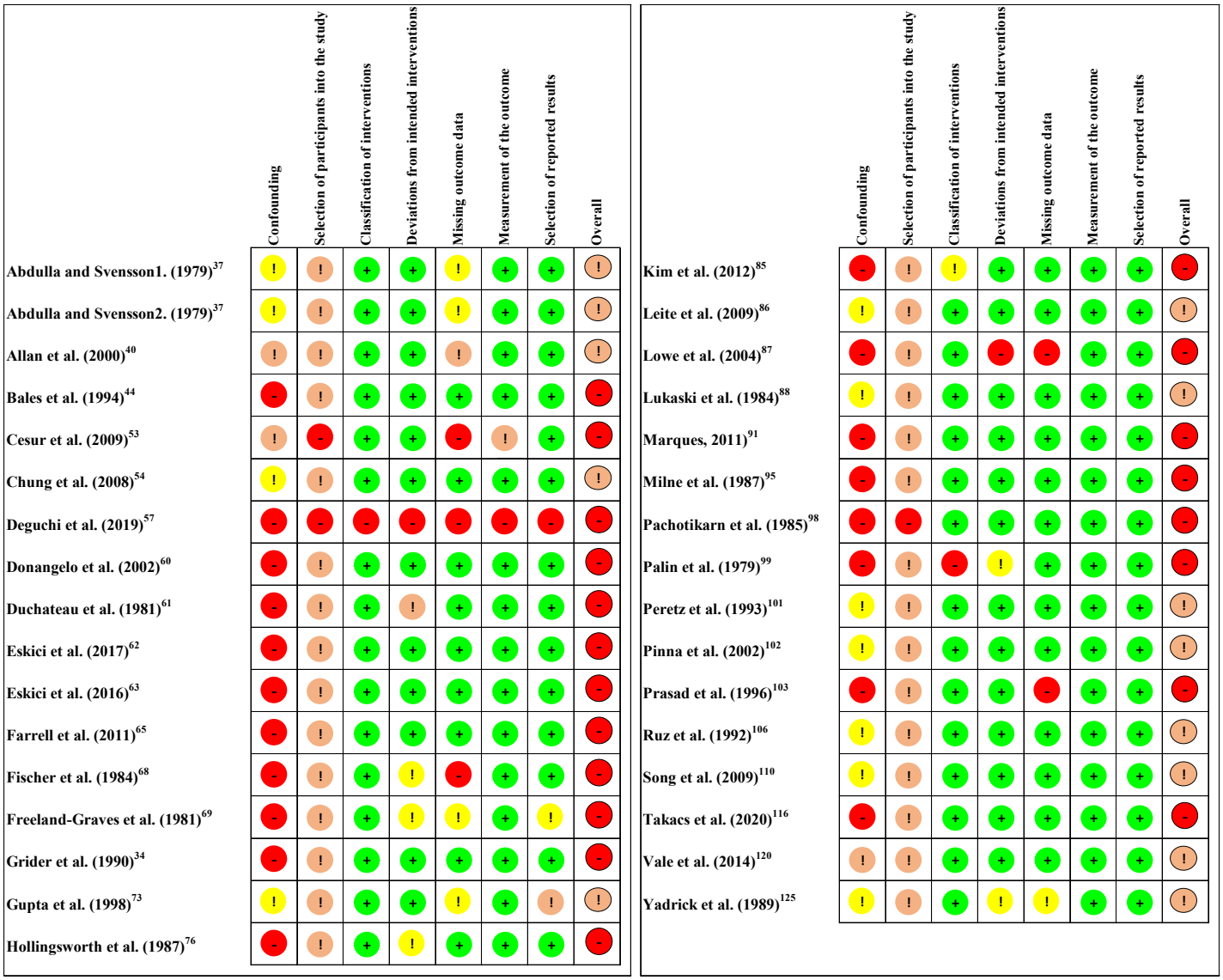


Figure 4. Risk of Bias summary of all non-randomized studies included in the narrative analysis, shown as the authors judgment for each ROBINS-I (Risk of bias in non-randomized studies of interventions) category for each study included.

# Risk of Bias Graphs

## Randomized control trials included in the meta-analysis

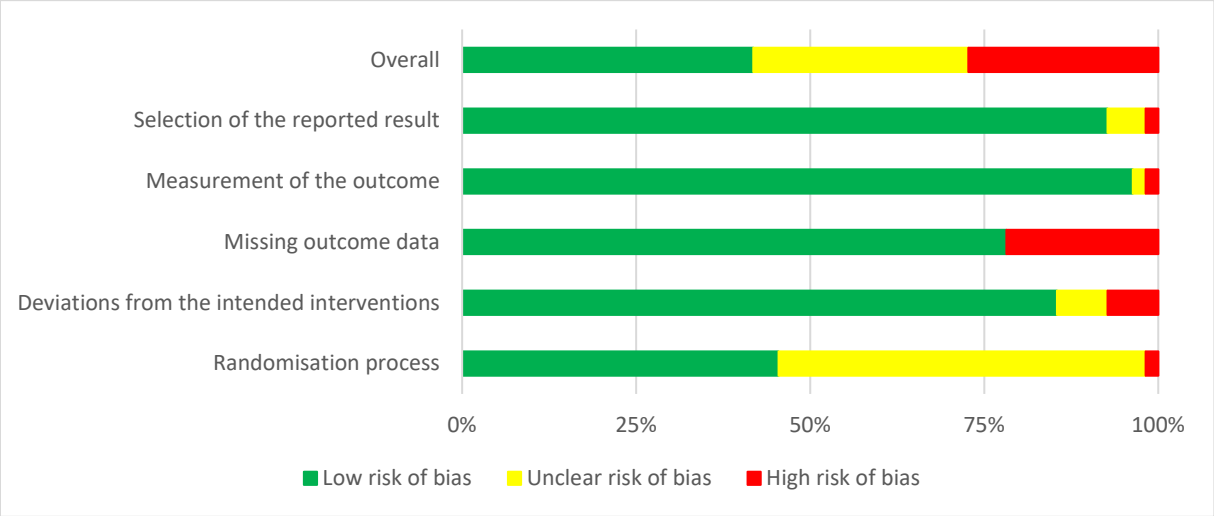


Figure 5. Risk of Bias graph of all randomized control trials included in the meta-analysis. Each risk of bias category is presented as a percentage of all the studies included in the meta-analysis, the overall bias is calculated as per the Cochrane RoB 2 algorithm (low risk if all categories are low risk, unclear risk if some categories have some concerns, and high risk if many categories have some concerns or if one or more categories has high risk).

## Non-randomized studies included in the meta-analysis

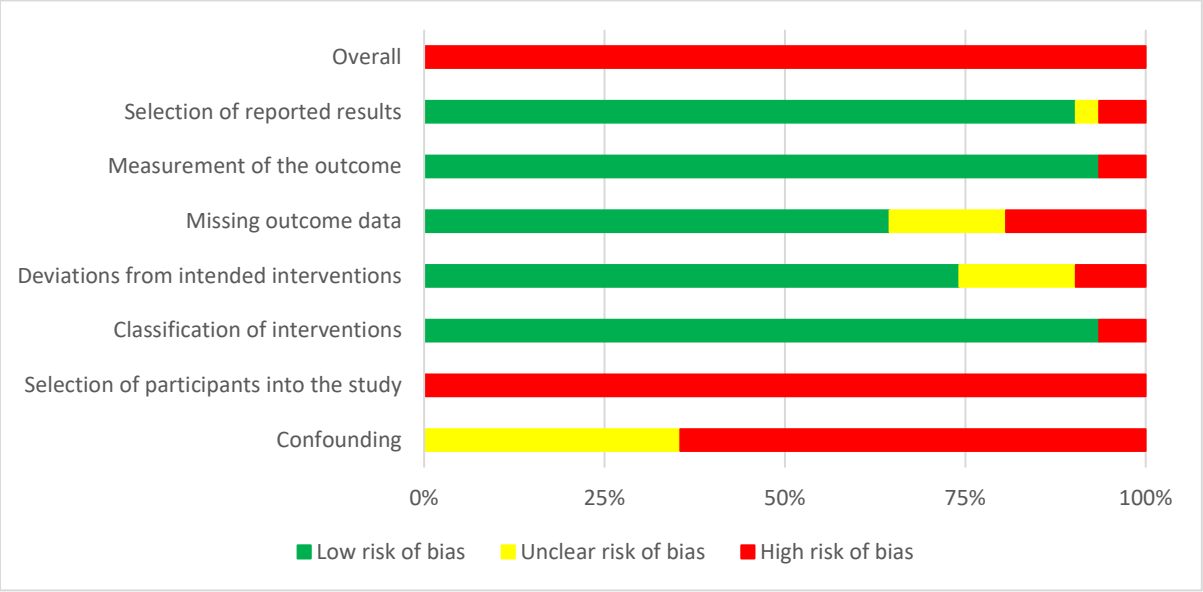


Figure 6. Risk of Bias graph of all non-randomized studies included in the meta-analysis. Each risk of bias category is presented as a percentage of all the studies included in the meta-analysis, the overall bias is calculated based on the same principles as the Cochrane RoB 2 algorithm (low risk if all categories are low risk, unclear risk if some categories have some concerns, and high risk if many categories have some concerns or if one or more categories has high risk).

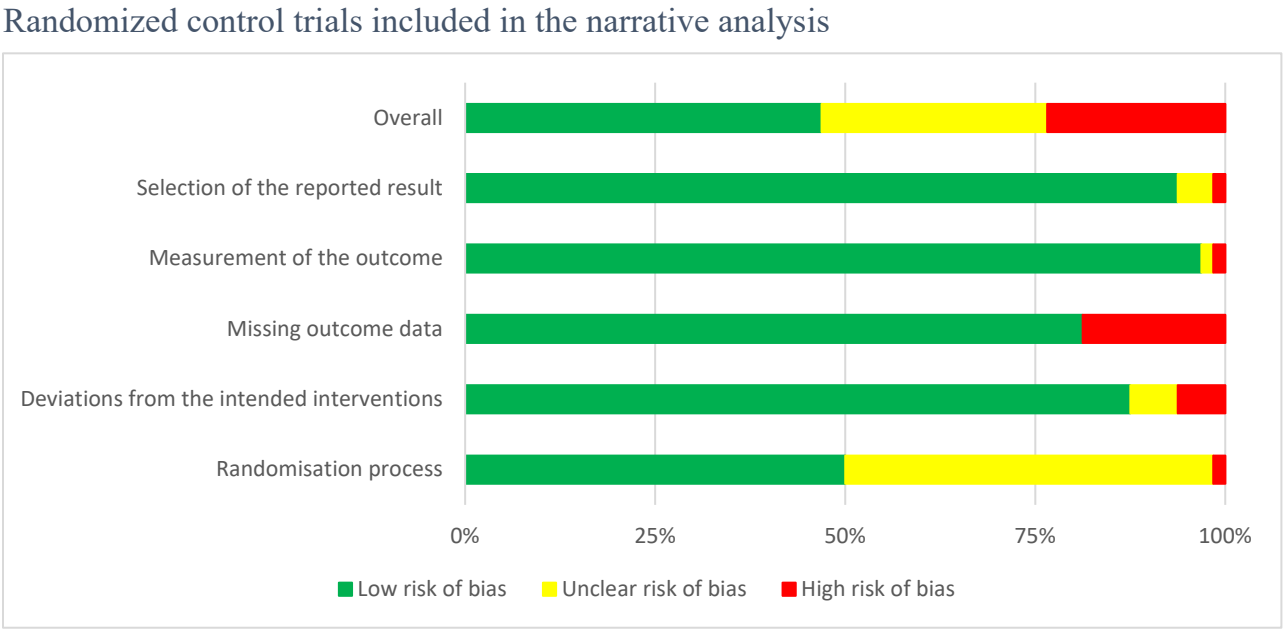


Figure 7. Risk of Bias graph of all randomized control trials included in the narrative analysis. Each risk of bias category is presented as a percentage of all the studies included in the narrative analysis, the overall bias is calculated as per the Cochrane RoB 2 algorithm (low risk if all categories are low risk, unclear risk if some categories have some concerns, and high risk if many categories have some concerns or if one or more categories has high risk).

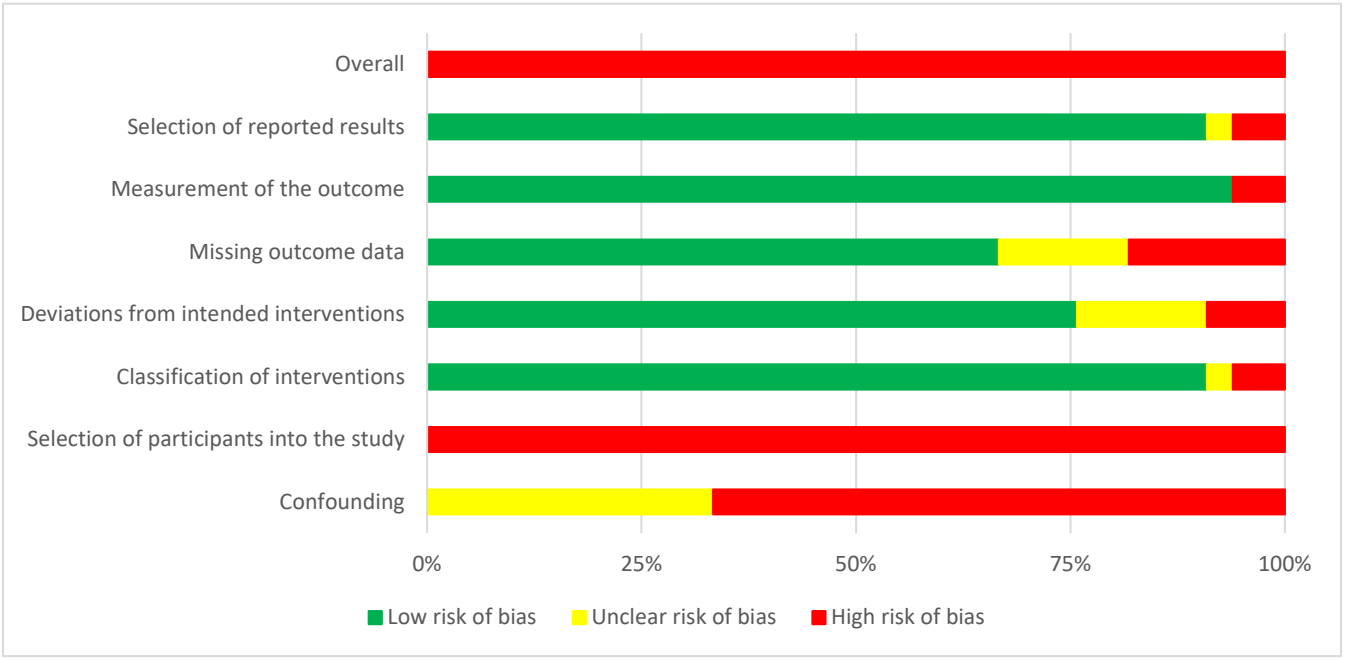


Figure 8. Risk of Bias graph of all non-randomized studies included in the narrative analysis. Each risk of bias category is presented as a percentage of all the studies included in the narrative analysis, the overall bias is calculated based on the same principles as the Cochrane RoB 2 algorithm (low risk if all categories are low risk, unclear risk if some categories have some concerns, and high risk if many categories have some concerns or if one or more categories has high risk).

# GRADE

## Grade Evidence table: Serum/Plasma zinc, controlled trials (mmol/L)

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Zinc supplement | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, controlled trials by study design: All studies** | | | | | | | | | | | | |
| 48 | RCTs / NRS | very serious[a] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 2223 | 2093 | - | MD **2.18 mmol/L higher** (1.74 higher to 2.61 higher) | ⨁◯◯◯ Very low | CRITICAL |
| **Serum/plasma zinc, controlled trials by study design: RCTs only** | | | | | | | | | | | | |
| 45 | RCTs | very serious[e] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 2196 | 2065 | - | MD **1.97 mmol/L higher** (1.55 higher to 2.4 higher) | ⨁◯◯◯ Very low | CRITICAL |
| **Serum/plasma zinc, controlled trials by study design: Non-randomised trials** | | | | | | | | | | | | |
| 3 | NRS | very serious[f] | serious[b] | not serious | serious[c] | none | 27 | 28 | - | MD **5.41 mmol/L higher** (2.42 lower to 13.23 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by sex: Males** | | | | | | | | | | | | |
| 8 | RCTs / NRS | very serious[g] | not serious | not serious | serious[c] | publication bias strongly suspected[d] | 138 | 114 | - | MD **1.67 mmol/L higher** (1.34 higher to 2.01 higher) | ⨁◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Zinc supplement | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, controlled trials by sex: Female** | | | | | | | | | | | | |
| 13 | RCTs | serious[h] | serious[i] | not serious | serious[c] | publication bias strongly suspected[d] | 516 | 502 | - | MD **1.58 mmol/L higher** (0.86 higher to 2.29 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by sex: Mixed male and female** | | | | | | | | | | | | |
| 26 | RCTs / NRS | serious[j] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 1569 | 1477 | - | MD **2.39 mmol/L higher** (1.84 higher to 2.94 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by population: Infants (0-12 months)** | | | | | | | | | | | | |
| 4 | RCTs | not serious | serious[k] | not serious | serious[l] | none | 157 | 180 | - | MD **2.72 mmol/L higher** (1.68 higher to 3.75 higher) | ⨁⨁◯◯ Low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by population: Children and adolescents** | | | | | | | | | | | | |
| 11 | RCTs | serious[m] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 882 | 907 | - | MD **0.96 mmol/L higher** (0.07 higher to 1.86 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by population: Pregnancy and lactation** | | | | | | | | | | | | |
| 3 | RCTs | serious[n] | serious[i] | not serious | serious[l] | none | 155 | 151 | - | MD **1.3 mmol/L higher** (0.09 higher to 2.7 higher) | ⨁◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Zinc supplement | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, controlled trials by population: Adults** | | | | | | | | | | | | |
| 23 | RCTs / NRS | serious[o] | serious[p] | not serious | serious[c] | publication bias strongly suspected[d] | 508 | 488 | - | MD **2.65 mmol/L higher** (1.8 higher to 3.5 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by population: Postmenopausal women** | | | | | | | | | | | | |
| 1 | RCTs | not serious | not serious | not serious | not serious | none | 57 | 55 | - | MD **4.64 mmol/L higher** (3.93 higher to 5.35 higher) | ⨁⨁⨁⨁ High | IMPORTANT |
| **Serum/plasma zinc, controlled trials by population: Elderly** | | | | | | | | | | | | |
| 4 | RCTs | very serious[q] | not serious | not serious | not serious | none | 147 | 120 | - | MD **3.54 mmol/L higher** (2.8 higher to 4.28 higher) | ⨁⨁◯◯ Low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by status at baseline: Normal serum/plasma zinc status at baseline** | | | | | | | | | | | | |
| 44 | RCTs / NRS | serious[r] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 1976 | 1868 | - | MD **2.15 mmol/L higher** (1.69 higher to 2.6 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by status at baseline: Low serum/plasma zinc status at baseline** | | | | | | | | | | | | |
| 4 | RCTs | serious[s] | serious[t] | not serious | serious[c] | none | 247 | 225 | - | MD **2.46 mmol/L higher** (0.9 higher to 4.01 higher) | ⨁◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Zinc supplement | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, controlled trials by dose: Supplement 1-2.9 mg Zn/d** | | | | | | | | | | | | |
| 2 | RCTs | serious[u] | not serious | not serious | not serious | none | 87 | 87 | - | MD **0.58 mmol/L higher** (0.37 lower to 1.54 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |
| **Serum/plasma zinc, controlled trials by dose: Supplementation 3 to 15 mg Zn/d** | | | | | | | | | | | | |
| 15 | RCTs | serious[v] | serious[b] | not serious | serious[b] | publication bias strongly suspected[d] | 1156 | 1121 | - | MD **2.05 mmol/L higher** (1.43 higher to 2.67 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by dose: Supplementation 16 to 25 mg Zn/d** | | | | | | | | | | | | |
| 10 | RCTs / NRS | serious[w] | serious[i] | not serious | serious[c] | publication bias strongly suspected[d] | 360 | 347 | - | MD **1.55 mmol/L higher** (0.68 higher to 2.42 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by dose: Supplementation 26 to 50 mg Zn/d** | | | | | | | | | | | | |
| 19 | RCTs / NRS | serious[x] | serious[y] | not serious | serious[c] | publication bias strongly suspected[d] | 544 | 484 | - | MD **1.9 mmol/L higher** (1.38 higher to 2.42 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by dose: Supplementation 51 to 100 mg Zn/d** | | | | | | | | | | | | |
| 4 | RCTs | very serious[z] | not serious | not serious | serious[c] | none | 56 | 37 | - | MD **4.16 mmol/L higher** (2.92 higher to 5.41 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Zinc supplement | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, controlled trials by dose: Supplementation 101 to 151 mg Zn/d** | | | | | | | | | | | | |
| 2 | RCTs / NRS | very serious[aa] | very serious[ab] | not serious | serious[c] | none | 20 | 17 | - | MD **7.55 mmol/L higher** (1.7 lower to 16.8 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by supplement type: Zinc sulphate** | | | | | | | | | | | | |
| 29 | RCTs / NRS | serious[ac] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 1526 | 1555 | - | MD **1.96 mmol/L higher** (1.38 higher to 2.54 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by supplement type: Zinc gluconate** | | | | | | | | | | | | |
| 17 | RCTs / NRS | serious[ad] | serious[ae] | not serious | serious[c] | publication bias strongly suspected[d] | 612 | 485 | - | MD **2.17 mmol/L higher** (1.55 higher to 2.8 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, controlled trials by supplement type: Zinc acetate** | | | | | | | | | | | | |
| 2 | RCTs / NRS | very serious[af] | not serious | not serious | not serious | none | 85 | 53 | - | MD **4.05 mmol/L higher** (3.2 higher to 4.9 higher) | ⊕⊕◯◯ Low | IMPORTANT |

**CI:** confidence interval; **MD:** mean difference; **RCT**: randomized control trial; **NRS**: non-randomized studies

## Explanations

a. 48 studied included in the analysis, 45 RCTs and 3 NRS. RCTs - One had high risk of bias and 22 had unclear risk of bias in the randomisation process (selection bias), two had high risk of bias in deviations from the intended interventions (performance bias), eight had high risk of bias in

missing outcome data (attrition bias), one high risk of bias in measurement of the outcome (detection bias), and one has high risk of bias in selection of the reported result (selective outcome reporting bias). NRS - Two had high risk of bias due to confounding, three had high risk of bias in selection of participants into the study, one had high risk of bias in classification of intervention (selection bias), two had unclear risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias and one had unclear risk of bias in missing outcome data (attrition bias). Overall, 14 had unclear risk of bias and 12 had high risk of bias.

b. Wide difference in point estimates, considerable heterogeneity, $I^2$ >95%.

c. Small number of events, wide confidence intervals including appreciable benefit and harm.

d. Publication bias suspected because of asymmetrical funnel plot.

e. 45 RCTs included in the analysis. One had high risk of bias and 22 had unclear risk of bias in the randomisation process (selection bias), two had high risk of bias in deviations from the intended interventions (performance bias), eight had high risk of bias in missing outcome data (attrition bias), one had high risk of bias in measurement of the outcome (detection bias), and one had high risk of bias in selection of the reported result (selective outcome reporting bias). Overall, 14 had unclear risk of bias and nine had high risk of bias.

f. Three studies included in the analysis. Two had high risk of bias due to confounding, three had high risk of bias in selection of participants into the study, one had high risk of bias in classification of intervention (selection bias), two had unclear risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias and one had unclear risk of bias in missing outcome data (attrition bias). Overall, three had high risk of bias.

g. Eight studies were included in the analysis, seven RCTs and one NRS. RCTs – One had high risk of bias and four had unclear risk of bias in the randomisation process (selection bias), two had high risk of bias in missing outcome data (attrition bias). NRS – High risk of bias due to confounding and selection of participants into the study (selection bias), unclear risk of bias in deviations from the intended interventions (performance bias), and high risk of bias in missing outcome data (attrition bias). Overall, 3 had high risk of bias and two had unclear risk of bias.

h. 13 RCTs were included in the analysis. Seven had unclear risk of bias in the randomisation process (selection bias), one had high risk of bias in deviations from the intended interventions (performance bias), two had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in measurement of the outcome (detection bias). Overall, two had high risk of bias and five had unclear risk of bias.

i. Considerable heterogeneity, $I^2$>95%.

j. 27 studies included in the analysis, 25 RCTs and two NRS. RCTs – 11 had unclear risk of bias in randomisation process (selection bias), one had high risk of bias in deviations from the intended interventions (performance bias), four had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in the selection of reported results (selective outcome reporting bias). NRS – One had high risk and one had unclear risk of confounding, two had high risk of bias in selection of participants into the study and one had high risk of bias in classification of interventions

(selection bias), one had unclear risk of bias in deviations from intended interventions (performance bias), and one had unclear risk of missing outcome data (attrition bias). Overall, seven had unclear risk of bias and seven had high risk of bias.

k. Considerable heterogeneity $I^2$ >90%.

l. Small sample size and small number of events.

m. 11 RCTs included in analysis. Two had high risk in missing outcome data (attrition bias), one high risk in measurement of the outcome (detection bias). Overall, two studies had high risk of bias.

n. 3 RCTs included in the analysis. Two had unclear risk of bias due to the randomisation process (selection bias), and one had unclear risk of bias in selection of the reported result (selective outcome reporting bias). Overall, two studies had unclear risk of bias.

o. 23 studies included in the analysis, 21 RCTs and two NRS. RCTs - One had high risk of bias and 10 had unclear risk of bias in the randomisation process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and four had high risk of bias in missing outcome data (attrition bias). NRS - One had high risk of bias and one had unclear risk of bias in confounding, two had high risk of bias in selection of participants into the study (selection bias), one had unclear risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias and one had unclear risk of bias in missing outcome data (attrition bias). Overall, six had high risk of bias and seven had unclear risk of bias.

p. Wide difference in point estimates, considerable heterogeneity, $I^2$ >90%.

q. Four RCTs included in the analysis. Four had unclear risk of bias for randomisation process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and two had high risk of bias in missing outcome data (attrition bias). Overall, two had high risk of bias and two had unclear rick of bias.

r. 44 studies included in analysis, 41 RCTs, three NRS. RCTs – One had high risk of bias and 20 had unclear risk of bias in the randomisation process (selection bias), two had high risk of bias in deviations from intended interventions (performance bias), eight had high risk of bias in missing outcome data (attrition bias), one had high risk of bias in measurement of the outcome (detection bias), and one had high risk of bias in selection of the reported result (selective outcome reporting bias). NRS – Two had high risk of bias and one had unclear risk of bias in confounding, three had high risk of bias in selection of participants into the study, and one had high risk of bias in classification intervention (selection bias), two had unclear risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, 12 had unclear risk of bias and 12 had high risk of bias.

s. Four RCTs included in the analysis. Two had unclear risk of bias in the randomisation process (selection bias). Overall, two studies had unclear risk of bias.

t. Considerable heterogeneity, $I^2$ >85%.

u. Two RCTs analysed. One had unclear risk of bias in randomisation process (selection bias), deviations from the intended interventions (performance bias), and high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias.

v. 15 RCTs analysed. One had high risk of bias in selection of the reported result (selective outcome reporting bias). Overall, one had high risk of bias.

w. Ten studies analysed, nine RCT and one NRS. RCTs- One had high risk of bias in missing outcome data (attrition bias), and one high risk of bias in measurement of the outcome (detection bias). NRS - High risk of bias in confounding, classification intervention, and selection of participants into the study (selection bias), unclear risk of bias in deviations from intended interventions (performance bias). Overall, two had high risk of bias.

x. 19 studies analysed, 18 RCTs and one NRS. RCTs – 11 had unclear risk of bias and one high risk of bias in randomisation process (selection bias), two had high risk of bias in deviations from the intended interventions (performance bias), five had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in selection of the reported results (selective outcome reporting bias). NRS - high risk of bias in confounding and selection of participants into the study (selection bias), unclear risk of bias in deviations from the intended interventions (performance bias), and high risk of bias in missing outcome data (attrition bias). Overall, six studies had high risk of bias and six studies unclear risk of bias.

y. Considerable heterogeneity, $I^2$>75%.

z. Four RCTs analysed. One had high risk of bias and three had unclear risk of bias in randomisation process (selection bias), one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias, three had unclear rick of bias.

aa. Two studies were analysed, one RCT and one NRS. RCT - Unclear risk of bias randomisation process (selection bias), and high risk of bias in missing outcome data (attrition bias). NRS - Unclear risk of bias in confounding and high risk of bias in deviations from the intended interventions (selection bias), unclear risk of bias in missing outcome data (attrition bias). Overall, two had high risk of bias.

ab. Wide difference in point estimates, confidence intervals do not overlap, considerable heterogeneity, $I^2$ >95%.

ac. 29 studies analysed, 27 RCTs and two NRS. RCTs – Four had high risk of bias in missing outcome data (attrition bias), one had high risk of bias in measurement of the outcome (detection bias). NRS – One had high risk of bias and one had unclear risk of bias in confounding, two had high risk of bias in selection of participants into the study, and one had had high risk of bias in classification of interventions (selection bias), one had unclear risk of bias in deviations from the intended interventions (performance bias), and one had unclear risk of bias in missing outcome data (attrition bias). Overall, seven had unclear risk of bias and six had high risk of bias.

ad. 17 studies analysed, 16RCT and one NRS. RCTs - One had high risk of bias and 10 had unclear risk of bias in the randomisation process (selection bias), one had high risk of bias in deviations from the intended interventions (performance bias), three had high risk of bias in missing outcome data (attrition bias), and one high risk of bias in selection of reported results (selective outcome reporting bias). NRS – High risk of bias in confounding, high risk of bias in selection of participants into the study (selection bias), unclear risk of bias in deviations from the intended

interventions (performance bias), and high risk of bias in missing outcome data (attrition bias). Overall, six studies had unclear risk of bias and five had high risk of bias.

ae. Wide difference in point estimates, considerable heterogeneity, $I^2$ >80%.

af. Two RCTs analysed. Two had unclear risk of bias in the randomisation process (selection bias), one high risk of bias in deviations from the intended interventions (performance bias), and one high risk of bias in missing outcome data (attrition bias). Overall, one had unclear risk of bias and one had high risk of bias.

## Grade Evidence table: Serum/Plasma zinc, before and after studies (mmol/L)

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | |
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by study design: All studies** | | | | | | | | | | | | |
| 79 | RCTs / NRS | very serious[a] | serious[b] | not serious | serious[c] | none | 2829 | 2931 | - | MD **2.85 mmol/L higher** (2.43 higher to 3.28 higher) | ⨁◯◯◯ Very low | CRITICAL |
| **Serum/plasma zinc, before and after studies by sex: Male** | | | | | | | | | | | | |
| 22 | RCTs / NRS | very serious[d] | serious[e] | not serious | serious[c] | none | 306 | 309 | - | MD **2.59 mmol/L higher** (1.85 higher to 3.33 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by sex: Female** | | | | | | | | | | | | |
| 22 | RCTs / NRS | very serious[f] | serious[b] | not serious | serious[c] | none | 664 | 665 | - | MD **2.82 mmol/L higher** (2.05 higher to 3.6 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by sex: Mixed male and female** | | | | | | | | | | | | |
| 37 | RCTs / NRS | very serious[g] | serious[b] | not serious | serious[c] | none | 1859 | 1957 | - | MD **2.96 mmol/L higher** (2.39 higher to 3.54 higher) | ⨁◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by population: Infants (0-12 months)** | | | | | | | | | | | | |
| 3 | RCTs | not serious | serious[h] | not serious | not serious | none | 157 | 174 | - | MD **2.8 mmol/L higher** (0.83 higher to 4.78 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |
| **Serum/plasma zinc, before and after studies by population: Children and adolescents** | | | | | | | | | | | | |
| 14 | RCTs / NRS | very serious[i] | serious[b] | not serious | serious[c] | publication bias strongly suspected[j] | 1127 | 1201 | - | MD **2.24 mmol/L higher** (1.38 higher to 3.09 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by population: Pregnancy and lactation** | | | | | | | | | | | | |
| 3 | RCTs | serious[k] | not serious | not serious | not serious | none | 155 | 155 | - | MD **0.82 mmol/L higher** (0.86 lower to 2.51 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |
| **Serum/plasma zinc, before and after studies by population: Adults** | | | | | | | | | | | | |
| 46 | RCTs / NRS | serious[l] | serious[m] | not serious | serious[c] | none | 865 | 872 | - | MD **3.28 mmol/L higher** (2.62 higher to 3.94 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by population: Post-menopausal women** | | | | | | | | | | | | |
| 1 | RCTs | not serious | not serious | not serious | not serious | none | 57 | 58 | - | MD **5.12 mmol/L higher** (4.42 higher to 5.82 higher) | ⊕⊕⊕⊕ High | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by population: Elderly** | | | | | | | | | | | | |
| 8 | RCTs / NRS | very serious[n] | serious[o] | not serious | serious[c] | none | 184 | 187 | - | MD **3.23 mmol/L higher** (2.31 higher to 4.16 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by status at baseline: Normal serum zinc level** | | | | | | | | | | | | |
| 74 | RCTs / NRS | very serious[p] | serious[b] | not serious | serious[c] | none | 2582 | 2681 | - | MD **2.87 mmol/L higher** (2.43 higher to 3.31 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by status at baseline: Low serum zinc level** | | | | | | | | | | | | |
| 4 | RCTs | serious[q] | serious[r] | not serious | serious[c] | none | 247 | 250 | - | MD **2.57 mmol/L higher** (0.89 higher to 4.26 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Depletion < 3 mg/d Zn** | | | | | | | | | | | | |
| 2 | NRS | very serious[s] | very serious[t] | not serious | serious[c] | none | 10 | 10 | - | MD **3.85 mmol/L higher** (5.65 higher to 13.36 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Depletion 3 to 15 mg/d Zn** | | | | | | | | | | | | |
| 9 | RCTs / NRS | very serious[u] | serious[v] | not serious | serious[c] | publication bias strongly suspected[j] | 78 | 78 | - | MD **1.42 mmol/L higher** (0.27 higher to 2.58 higher) | ⨁◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 1 to 2.9 mg/d Zn** | | | | | | | | | | | | |
| 2 | RCTs | very serious[w] | not serious | not serious | not serious | none | 87 | 87 | - | MD **1.05 mmol/L higher** (0.3 higher to 1.79 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 3 to 15 mg/d Zn** | | | | | | | | | | | | |
| 18 | RCTs/ NRS | serious[x] | serious[b] | not serious | serious[c] | publication bias strongly suspected[j] | 1331 | 1422 | - | MD **2.09 mmol/L higher** (1.46 higher to 2.73 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 16 to 25 mg/d Zn** | | | | | | | | | | | | |
| 13 | RCTs/ NRS | serious[y] | serious[b] | not serious | serious[c] | publication bias strongly suspected[j] | 411 | 412 | - | MD **1.74 mmol/L higher** (0.92 higher to 2.57 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 26 to 50 mg/d Zn** | | | | | | | | | | | | |
| 27 | RCTs / NRS | very serious[z] | serious[m] | not serious | serious[c] | none | 662 | 665 | - | MD **3.23 mmol/L higher** (2.43 higher to 4.02 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 51 to 100 mg/d Zn** | | | | | | | | | | | | |
| 8 | RCTs / NRS | very serious[aa] | serious[b] | not serious | serious[e] | publication bias strongly suspected[j] | 84 | 84 | - | MD **5.19 mmol/L higher** (1.81 higher to 8.58 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by dose: Supplementation 101 to 151 mg/d Zn** | | | | | | | | | | | | |
| 7 | RCTs / NRS | extremely serious[ab] | serious[e] | not serious | serious[c] | publication bias strongly suspected[j] | 166 | 173 | - | MD **5.46 mmol/L higher** (2.04 higher to 8.89 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by supplement type: Zinc Sulphate** | | | | | | | | | | | | |
| 39 | RCTs / NRS | very serious[ac] | serious[b] | not serious | serious[c] | none | 1919 | 2018 | - | MD **3.22 mmol/L higher** (2.59 higher to 3.85 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by supplement type: Zinc gluconate** | | | | | | | | | | | | |
| 24 | RCTs / NRS | very serious[ad] | serious[e] | not serious | serious[c] | publication bias strongly suspected[j] | 706 | 709 | - | MD **2.56 mmol/L higher** (1.94 higher to 3.18 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by supplement type: Zinc acetate** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[ae] | not serious | not serious | not serious | none | 94 | 94 | - | MD **3.6 mmol/L higher** (2.87 higher to 4.33 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Serum/plasma zinc, before and after studies by supplement type: Depletion** | | | | | | | | | | | | |
| 11 | RCTs / NRS | extremely serious[af] | serious[b] | not serious | serious[e] | publication bias strongly suspected[j] | 88 | 88 | - | MD **1.88 mmol/L higher** (0.39 higher to 3.37 higher) | ⊕◯◯◯ Very low | IMPORTANT |

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| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Before | After | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum/plasma zinc, before and after studies by supplement type: Mixed zinc gluconate and zinc acetate** | | | | | | | | | | | | |
| 1 | NRS | very serious[ag] | not serious | not serious | not serious | none | 22 | 22 | - | MD **2.53 mmol/L higher** (0.45 higher to 4.62 higher) | ⊕⊕◯◯ Low | IMPORTANT |

**CI:** confidence interval; **MD:** mean difference**; RCT**: randomized control trial; **NRS**: non-randomized studies

## Explanations

a. 49 RCTs and 28 NRS and 2 studies not available for RoB assessment. RCTs-High risk of bias in the randomization process (selection bias), deviations from intended interventions (performance bias), missing outcome data (attrition bias), measurement of the outcome (detection bias), selection of the reported results (selective outcome reporting bias). NRS–high risk of bias in confounding, selection of participants into the study, classification of the interventions (selection bias), deviations from intended interventions (performance bias), missing outcome data (attrition bias), measurement of the outcome (detection bias), selection of the reported results (selective outcome reporting bias). Overall, 40/79 had high risk of bias.

b. Wide difference in point estimates, considerable heterogeneity, $I^2$ >95%.

c. Small number of events, wide confidence intervals including appreciable benefit and harm.

d. 22 studies included in the analysis, 11 RCTs, 10 NRS, 1 study was not available for RoB. RCTs – 5 studies had unclear risk of bias and 1 had high risk of bias in randomization process (selection bias), 2 had high risk of bias in deviations from intended interventions (performance bias), and 2 had high risk of bias in missing outcome data (attrition bias). NRS - 6 had high risk of bias in confounding, 10 had high risk of bias in selection of participants into the study (selection bias), 2 had high risk of bias in deviations from intended interventions (performance bias), and 3 had high risk of bias in missing outcome data (attrition bias). Overall, 14 had high risk of bias.

e. Wide difference in point estimates, considerable heterogeneity, $I^2$ >90%.

f. 22 studies included in the analysis, 15 RCTs and 7 NRS. RCTs – 8 had unclear risk of bias in randomization process (selection bias), 2 had high risk of bias in deviations from intended interventions (performance bias), 3 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in measurement of the outcome (detection bias). NRS - 6 had high risk of bias in confounding, 7 had high risk of bias in selection of participants into the study (selection bias), and 1 had high risk of bias in in deviations from intended interventions (performance bias). Overall, 10 had high risk of bias and 6 had unclear risk of bias.

g. 37 studies analysed, 24 RCTs, 12 NRS, 1 study not available for RoB. RCTs- 2 had unclear risk of bias in randomization process (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 5 had had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS–7 had high risk of bias in confounding, 12 had high risk of bias in selection of participants into the study and 2 had high risk of bias in classification of the interventions (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 2 had high risk of bias in missing outcome data (attrition bias), 2 had high risk of bias in measurement of the outcome (detection bias), and 2 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 18 had high risk of bias and 7 had unclear risk of bias.

h. Considerable heterogeneity, $I^2$ >95%.

i. 14 papers were in included in the analysis, 11 RCTs and 3 NRS. RCTs – 2 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in measurement of the outcome (detection bias). NRS – 2 had high risk of bias in confounding and 3 had high risk of bias in selection of participants into the study (selection bias), 1 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in measurement of the outcome (detection bias). Overall, 5 had high risk of bias and 3 had unclear risk of bias.

j. Publication bias suspected because of asymmetrical funnel plot.

k. 3 RCTs were included in the analysis. 2 had unclear risk of bias in randomization process (selection bias). Overall, 2 had unclear risk of bias.

l. 46 papers were included in the analysis, 25 RCTs, 18 NRS, 2 papers were unavailable for RoB. RCTs – 12 had unclear risk of bias and 1 had high risk of bias in randomization process (selection bias). 3 had high risk of bias in deviations from intended interventions (performance bias), and 5 had high risk of bias in missing outcome data (attrition bias). NRS - 10 had high risk of bias and 9 had unclear risk of bias in confounding, 19 had high risk of bias in selection of participants into the study (selection bias), 2 had high risk of bias in deviations from intended interventions (performance bias), 2 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 26 had high risk of bias and 8 had unclear risk of bias.

m. Wide difference in point estimates, considerable heterogeneity, $I^2$ >90%.

n. 8 papers were included in the analysis, 5 RCTs and 3 NRS. RCTs – 5 had unclear risk of bias in the randomization process (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), and 3 had high risk of bias in missing outcome data (attrition bias). NRS – 3 had high risk of bias in confounding, 3 had high risk of bias in selection of participants into the study, and 1 had high risk of bias in

classification of the interventions (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 2 had high risk of bias in missing outcome data (attrition bias), 1 had high risk of bias in measurement of the outcome (detection bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 6 had high risk of bias.

o. Considerable heterogeneity, $I^2$ >55%.

p. 74 papers analysed, 45 RCTs, 27 NRS, 2 papers unavailable for RoB. RCTs–High risk of bias in the randomization process (selection bias), high risk of bias in deviations from intended interventions (performance bias), high risk of bias in missing outcome data (attrition bias), high risk of bias in measurement of the outcome (detection bias), high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS-high risk of bias in confounding, selection of participants into the study and classification of the interventions (selection bias), high risk of bias in deviations from intended interventions (performance bias), high risk of bias in missing outcome data (attrition bias), high risk of bias in measurement of the outcome (detection bias), high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 40 papers had high risk of bias.

q. 4 RCTs included in the analysis. 2 had unclear risk of bias the randomization process (selection bias). Overall, 2 had unclear risk of bias.

r. Considerable heterogeneity, $I^2$ >90%.

s. 2 NRS included in the analysis. 2 had high risk of bias in confounding and selection of participants into the study (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), and 1 had high risk of bias in missing outcome data (attrition bias). Overall, 2 had high risk of bias.

t. Wide difference in point estimates, confidence intervals do not overlap, considerable heterogeneity, $I^2$ >95%.

u. 9 papers were included in the analysis, 1 RCT and 7 NRS, 1 paper was unavailable for RoB. RCT – Unclear risk of bias in the randomization process (selection bias). NRS – 4 had unclear risk of bias and 3 had high risk of bias in confounding, 7 had high risk of bias in selection of participants into the study (selection bias), and 1 had high risk of bias in missing outcome data (attrition bias). Overall, 1 had unclear risk of bias and 7 had high risk of bias.

v. Wide difference in point estimates, considerable heterogeneity, $I^2$ >85%.

w. 2 RCTs were included in the analysis. 1 had unclear risk of bias in the randomization process (selection bias), 1 had unclear risk of bias in deviations from intended interventions (performance bias), and 1 high risk of bias in in missing outcome data (attrition bias). Overall, 1 had high risk of bias.

x. 18 papers were included in the analysis, 15 RCTs and 3 NRS. RCTs – 1 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – 2 had high risk of bias in confounding, and 3 had high risk of bias in selection of participants into the study (selection bias). Over all 5 had high risk of bias.

y. 13 papers were included in the analysis, 10 RCTs and 3 NRS. RCTs - 1 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in measurement of the outcome (detection bias). NRS – 3 had high risk of bias in confounding, 3 had high risk of bias in selection of participants into the study, and 1 had high risk of bias in classification of the interventions (selection bias). Overall, 4 had high risk of bias.

z. 27 papers analysed, 20 RCTs and 7 NRS. RCTs – 1 had high risk of bias in the randomization process (selection bias), 3 had high risk of bias in deviations from intended interventions (performance bias), 7 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS - 5 had high risk of bias in confounding, 7 had high risk of bias in selection of participants into the study, and 1 had high risk of bias in classification of the interventions (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 3 had high risk of bias in missing outcome data (attrition bias), 2 had high risk of bias in measurement of the outcome (detection bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 16 had high risk of bias.

aa. 8 papers were included in the analysis, 5 RCTs and 3 NRS. RCTs – 4 had unclear risk of bias and 1 had high risk of bias in the randomization process (selection bias), and 2 had high risk of bias in missing outcome data (attrition bias). NRS - 3 had high risk of bias in confounding and 3 had high risk of bias in selection of participants into the study (selection bias). Overall, 5 had high risk of bias.

ab. 7 papers were included in the analysis, 3 RCTs and 3 NRS, 1 paper was unavailable for RoB. RCTs – 2 had unclear risk of bias in the randomization process (selection bias), 1 had unclear risk of bias and 1 had high risk of bias in deviations from intended interventions (performance bias), and 2 had high risk of bias in missing outcome data (attrition bias). NRS – 2 had unclear risk of bias and 1 had high risk of bias in confounding, and 3 had high risk of bias in selection of participants into the study (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 2 had unclear risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 6 papers had high risk of bias.

ac. 39 papers analysed, 29 RCTs and 9 NRS, 1 paper unavailable for RoB. RCTs–11 had unclear risk of bias in the randomization process (selection bias), 2 had high risk of bias in deviations from intended interventions (performance bias), 5 had high risk of bias in missing outcome data (attrition bias), and 1 had high risk of bias in measurement of the outcome (detection bias). NRS-6 had high risk of bias in confounding, 9 had high risk of bias in selection of participants into the study, and 1 had high risk of bias in classification of the interventions (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), 1 had high risk of bias in missing outcome data (attrition bias), 1 had high risk of bias in measurement of the outcome (detection bias), and 1 had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, 16 had high risk of bias.

ad. 24 papers were included in the analysis, 17 RCTs and seven NRS. RCTs - 11 had unclear risk of bias and one had high risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), four had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS - Two had unclear risk of bias and five had high risk of bias in confounding, seven had high risk of bias in selection of participants into the

study (selection bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, 12 had high risk of bias and six had unclear risk of bias.

ae. 3 papers were included in the analysis, 2 RCTs and 1 NRS. RCTs – 2 had unclear bias in the randomization process (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), and 1 had high risk of bias in missing outcome data (attrition bias). NRS – High risk of bias in confounding, selection of participants into the study and classification of the interventions (selection bias), deviations from intended interventions (performance bias), missing outcome data (attrition bias), measurement of the outcome (detection bias), and the selection of the reported results (selective outcome reporting bias). Overall, 2 had high risk of bias and 1 had unclear risk of bias.

af. 11 papers were included in the analysis, 1 RCT and 9 NRS, 1 paper was unavailable for RoB. RCT – Unclear risk of bias in the randomization process (selection bias). NRS – 4 had unclear risk of bias and 5 had high risk of bias in confounding, and 9 had high risk of bias in selection of participants into the study (selection bias), 1 had high risk of bias in deviations from intended interventions (performance bias), and 2 had high risk of bias in missing outcome data (attrition bias). Overall, 9 had high risk of bias.

ag. 1 NRS. High risk of bias in confounding and selection of participants into the study (selection bias).

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## Grade Evidence table: Urinary zinc

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (mmol/mol Creatinine): All studies** | | | | | | | | | | | | |
| 4 | RCTs / NRS | very serious[a] | serious[b] | not serious | serious[c] | none | 311 | 176 | - | MD **0.39 mmol/mol Creatinine higher** (0.17 higher to 0.62 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Urinary Zinc (mmol/mol Creatinine) by sex: Males** | | | | | | | | | | | | |
| 2 | RCTs | serious[d] | not serious | not serious | serious[c] | none | 43 | 35 | - | MD **0.71 mmol/mol Creatinine higher** (0.53 higher to 0.89 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by sex: Females** | | | | | | | | | | | | |
| 1 | NRS | very serious[e] | not serious | not serious | serious[c] | none | 11 | 11 | - | MD **0.27 mmol/mol Creatinine higher** (0.02 higher to 0.52 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by sex: Mixed males and females** | | | | | | | | | | | | |
| 1 | RCT | very serious[f] | not serious | not serious | serious[c] | none | 257 | 130 | - | MD **0.21 mmol/mol Creatinine higher** (0.03 higher to 0.4 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by population: Children and adolescents** | | | | | | | | | | | | |
| 1 | RCT | not serious | serious[g] | not serious | not serious | none | 21 | 26 | - | MD **0.77 mmol/mol Creatinine higher** (0.56 higher to 0.98 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (mmol/mol Creatinine) by population: Adults** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[h] | serious[i] | not serious | serious[c] | none | 290 | 150 | - | MD **0.25 mmol/mol Creatinine higher** (0.13 higher to 0.37 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by dose: Supplementation 15 to 25 mg Zn/d** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[j] | serious[k] | not serious | serious[c] | none | 158 | 102 | - | MD **0.38 mmol/mol Creatinine higher** (0.03 lower to 0.79 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by dose: Supplementation 26 to 50 mg Zn/d** | | | | | | | | | | | | |
| 2 | RCTs | very serious[l] | not serious | not serious | serious[c] | none | 144 | 70 | - | MD **0.32 mmol/mol Creatinine higher** (0.18 higher to 0.47 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by dose: Supplementation 51 to 100 mg Zn/d** | | | | | | | | | | | | |
| 1 | RCT | very serious[m] | not serious | not serious | serious[c] | none | 9 | 4 | - | MD **0.59 mmol/mol Creatinine higher** (0.04 lower to 1.22 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (mmol/mol Creatinine) by supplement type: Zinc gluconate** | | | | | | | | | | | | |
| 4 | RCTs / NRS | very serious[a] | serious[b] | not serious | serious[c] | none | 311 | 176 | - | MD **0.39 mmol/mol Creatinine higher** (0.17 higher to 0.62 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (µmol/d): All studies** | | | | | | | | | | | | |
| 6 | RCTs / NRS | very serious[n] | serious[k] | not serious | serious[c] | publication bias strongly suspected[o] | 71 | 64 | - | MD **3.09 µmol/d higher** (0.16 higher to 6.02 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by sex: Males** | | | | | | | | | | | | |
| 4 | RCTs / NRS | very serious[p] | serious[k] | not serious | serious[c] | none | 36 | 33 | - | MD **3.87 µmol/d higher** (0.25 higher to 7.49 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by sex: Females** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[q] | serious[r] | not serious | serious[c] | none | 35 | 31 | - | MD **2.99 µmol/d higher** (0.7 lower to 6.67 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by population: Adults** | | | | | | | | | | | | |
| 4 | RCTs / NRS | very serious[s] | serious[k] | not serious | serious[c] | none | 49 | 49 | - | MD **2.5 µmol/d higher** (1.01 lower to 6 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by population: Elderly** | | | | | | | | | | | | |
| 1 | RCT | very serious[t] | not serious | not serious | not serious | none | 17 | 10 | - | MD **9.3 µmol/d higher** (5.98 higher to 12.62 higher) | ⊕⊕◯◯ Low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (µmol/d) by dose: Depletion <5 mg Zn/d** | | | | | | | | | | | | |
| 4 | RCTs / NRS | very serious[u] | serious[k] | not serious | serious[c] | none | 29 | 29 | - | MD **2.98 µmol/d higher** (0.48 lower to 6.43 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by dose: Supplementation 15 to 25 mg Zn/d** | | | | | | | | | | | | |
| 1 | RCT | serious[v] | not serious | not serious | not serious | none | 5 | 5 | - | MD **0.3 µmol/d lower** (2.11 lower to 1.51 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |
| **Urinary Zinc (µmol/d) by dose: Supplementation 26 to 50 mg Zn/d** | | | | | | | | | | | | |
| 2 | RCTs | very serious[w] | very serious[x] | not serious | serious[c] | none | 37 | 30 | - | MD **5.31 µmol/d higher** (2.41 lower to 13.03 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/d) by supplement type: Zn sulphate** | | | | | | | | | | | | |
| 1 | RCT | serious[v] | not serious | not serious | not serious | none | 5 | 5 | - | MD **0.3 µmol/d lower** (2.11 lower to 1.51 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |
| **Urinary Zinc (µmol/d) by supplement type: Zn gluconate** | | | | | | | | | | | | |
| 1 | RCT | very serious[t] | not serious | not serious | serious[y] | none | 20 | 20 | - | MD **1.42 µmol/d higher** (1.44 lower to 4.28 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (µmol/d) by supplement type: Zn acetate** | | | | | | | | | | | | |
| 1 | RCT | serious[t] | not serious | not serious | serious[z] | none | 17 | 10 | - | MD **9.3 µmol/d higher** (5.98 higher to 12.62 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Urinary Zinc (µmol/L): All studies** | | | | | | | | | | | | |
| 4 | RCTs / NRS | serious[aa] | serious[k] | not serious | serious[c] | none | 63 | 64 | - | MD **2.88 µmol/L higher** (1.55 lower to 7.31 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/L) by sex: Males** | | | | | | | | | | | | |
| 1 | NRS | very serious[ab] | not serious | not serious | serious[y] | none | 14 | 15 | - | MD **1.6 µmol/L lower** (9.29 lower to 6.09 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/L) by sex: Females** | | | | | | | | | | | | |
| 2 | RCTs / NRS | serious[ac] | very serious[x] | not serious | serious[ad] | none | 34 | 34 | - | MD **4.38 µmol/L higher** (2.49 lower to 11.25 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/L) by sex: Mixed** | | | | | | | | | | | | |
| 1 | RCT | not serious | not serious | not serious | serious[y] | none | 15 | 15 | - | MD **2.29 µmol/L higher** (0.35 higher to 4.23 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (µmol/L) by population: Children and adolescents** | | | | | | | | | | | | |
| 1 | NRS | very serious[e] | not serious | not serious | not serious | none | 10 | 10 | - | MD **7.87 µmol/L higher** (6.79 higher to 8.96 higher) | ⨁⨁◯◯ Low | IMPORTANT |
| **Urinary Zinc (µmol/L) by population: Adults** | | | | | | | | | | | | |
| 3 | RCTs / NRS | serious[ae] | not serious | not serious | serious[c] | none | 53 | 54 | - | MD **1.28 µmol/L higher** (0.16 higher to 2.39 higher) | ⨁⨁◯◯ Low | IMPORTANT |
| **Urinary Zinc (µmol/L) by dose: Depletion <5 mg Zn/d** | | | | | | | | | | | | |
| 1 | NRS | very serious[ab] | not serious | not serious | serious[y] | none | 14 | 15 | - | MD **1.6 µmol/L higher** (9.29 lower to 6.09 higher) | ⨁◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/L) by dose: Supplementation 15 to 25 mg Zn/d** | | | | | | | | | | | | |
| 1 | RCT | not serious | not serious | not serious | not serious | none | 24 | 24 | - | MD **0.86 µmol/L higher** (0.52 lower to 2.24 higher) | ⨁⨁⨁⨁ High | IMPORTANT |
| **Urinary Zinc (µmol/L) by dose: Supplementation 26 to 50 mg Zn/d** | | | | | | | | | | | | |
| 2 | RCTs / NRS | serious[ac] | serious[k] | not serious | serious[ad] | none | 25 | 25 | - | MD **5.14 µmol/L higher** (0.33 lower to 10.61 higher) | ⨁◯◯◯ Very low | IMPORTANT |

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| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Urinary zinc | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Urinary Zinc (µmol/L) by supplement type: Zinc sulphate** | | | | | | | | | | | | |
| 2 | RCTs / NRS | serious[ac] | very serious[x] | not serious | serious[ad] | none | 34 | 34 | - | MD **4.38 µmol/L higher** (2.49 lower to 11.25 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Urinary Zinc (µmol/L) by supplement type: Zinc gluconate** | | | | | | | | | | | | |
| 1 | RCT | not serious | not serious | not serious | serious[y] | none | 15 | 15 | - | MD **2.29 µmol/L higher** (0.35 higher to 4.23 higher) | ⊕⊕⊕◯ Moderate | IMPORTANT |

**CI:** confidence interval; **MD:** mean difference; **RCT**: randomized control trial; **NRS**: non-randomized studies

## Explanations

a. Four studies were included in the analysis, three RCTs and 1 NRS. RCTs - One had high risk of bias and one had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – High risk of bias in confounding, and selection of participants into the study (selection bias). Overall, three had high risk of bias.

b. Wide difference in point estimates, considerable heterogeneity, $I^2 > 80\%$.

c. Small number of events, wide confidence intervals including appreciable benefit and harm.

d. Two RCTs were included in the analysis. One had high risk of bias in the randomization process (selection bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias.

e. One NRS included in the analysis – High risk of bias in confounding, and high risk of bias in selection of participants into the study (selection bias).

f. One RCT paper only. Unclear risk of bias in the randomization process (selection bias), high risk of bias in the selection of the reported results (selective outcome reporting bias).

g. $I^2 > 100\%$.

h. Three studies included in the analysis, two RCTs and one NRS. RCTs- One had high risk of bias and one had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – High risk of bias in confounding, and selection of participants into the study (selection bias). Overall, three had high risk of bias.

i. Wide difference in point estimates.

j. Three studies included in the analysis, two RCTs and one NRS. RCTs- One had unclear risk of bias in the randomization process (selection bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS - High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias.

k. Wide difference in point estimates, considerable heterogeneity, $I^2 > 90\%$.

l. Two RCTs were included in the analysis. One had high risk of bias and one had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in missing outcome data (attrition bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, two had high risk of bias.

m. One RCTs included in the analysis. High risk of bias in the randomization process (selection bias), and high risk of bias in missing outcome data (attrition bias).

n. Six studies were included in the analysis, three RCTs and three NRS. RCTs- Three had unclear risk of bias in the randomization process (selection bias), two had high risk of bias in deviations from intended interventions (performance bias), and two had high risk of bias in missing outcome data (attrition bias). NRS – One had unclear risk of bias and two had high risk of bias in confounding, and three had high risk of bias in selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, five had high risk of bias and one had unclear risk of bias.

o. Publication bias suspected because of asymmetrical funnel plot.

p. Four studies were included in the analysis, two RCTs and two NRS. RCTs - Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). NRS – One had unclear risk of bias and one had high risk of bias in confounding, and two had high risk of bias in selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, all three had high risk of bias and one had unclear risk of bias.

q. Three studies were included in the analysis, two RCTs and one NRS. RCTs. Two had unclear risk of bias in the randomization process (selection bias), two had high risk of bias in deviations from intended interventions (performance bias), and two had high risk of bias in missing outcome data (attrition bias). NRS – High risk of bias in confounding and selection of participants into the study (selection bias). Overall, all three had high risk of bias.

r. Wide difference in point estimates, considerable heterogeneity, $I^2 > 75\%$.

s. Four studies were included in the analysis, two RCTs and two NRS. RCTs- Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). NRS – One had unclear risk of bias and one had high risk of bias in confounding, and two had high risk of bias in selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, three had high risk of bias and one had unclear risk of bias.

t. One RCT included in the analysis. Unclear risk of bias in the randomization process (selection bias), high risk of bias in deviations from intended interventions (performance bias), and high risk of bias in missing outcome data (attrition bias).

u. Four studies were included in the analysis, one RCTs and three NRS. RCTs - One had unclear risk of bias in the randomization process (selection bias). NRS – One had unclear risk of bias and two had high risk of bias in confounding, and three had high risk of bias in selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, three had high risk of bias and one had unclear risk of bias.

v. One RCT included in the analysis. Unclear risk of bias in the randomization process (selection bias).

w. Two RCTs were included in the analysis. Two had unclear risk of bias in the randomization process (selection bias), two had high risk of bias in deviations from intended interventions (performance bias), and two had high risk of bias in missing outcome data (attrition bias). Overall, two had high risk of bias.

x. Wide difference in point estimates, confidence intervals do not overlap, considerable heterogeneity, $I^2 > 90\%$.

y. Wide confidence intervals including appreciable benefit and harm.

z. Wide confidence intervals.

aa. Four studies were included in the analysis, two RCTs and two NRS. RCTs - Both at low risk. NRS – One had unclear risk of bias and one had high risk of bias in confounding, and two had high risk of bias in selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

ab. One NRS included in the analysis. Unclear risk of bias in confounding, and high risk of bias in selection of participants into the study (selection bias).

ac. Two studies were included in the analysis, one RCT and one NRS. RCT at low risk of bias. NRS – High risk of bias in confounding and selection of participants into the study (selection bias). Overall, one had high risk of bias

ad. Small number of events, wide estimate points indicate appreciable benefit and harm..

ae. Three studies were included in the analysis, two RCTs and one NRS. RCTs - Both at low risk. NRS –Unclear risk of bias in confounding and high risk of bias in selection of participants into the study (selection bias). Overall, one had high risk of bias.

## Grade Evidence table: Alkaline phosphatase (ALP; U/L)

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | ALP | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Alkaline phosphatase (U/L): All studies** | | | | | | | | | | | | |
| 7 | RCTs / NRS | very serious[a] | serious[b] | not serious | serious[c] | publication bias strongly suspected[d] | 364 | 237 | - | MD **3.88 higher** (0.43 higher to 7.33 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Alkaline phosphatase (U/L) by sex: Males** | | | | | | | | | | | | |
| 1 | NRS | very serious[e] | not serious | not serious | not serious | none | 5 | 5 | - | MD **21.8 higher** (8.91 higher to 34.69 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Alkaline phosphatase (U/L) by sex: Female** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[f] | not serious | not serious | serious[c] | none | 55 | 55 | - | MD **5.44 higher** (1.38 lower to 12.25 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Alkaline phosphatase (U/L) by sex: Mixed male and female** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[g] | not serious | not serious | serious[c] | none | 304 | 177 | - | MD **1.72 higher** (0.14 higher to 3.3 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Alkaline phosphatase (U/L) by intake: Depletion < 3 mg/d Zn** | | | | | | | | | | | | |
| 2 | NRS | very serious[h] | serious[i] | not serious | serious[c] | none | 10 | 10 | - | MD **12.17 higher** (6.47 lower to 31.09 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | ALP | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Alkaline phosphatase (U/L) by intake: Supplementation 3 to 15 mg/d Zn** | | | | | | | | | | | | |
| 2 | RCTs / NRS | very serious[j] | not serious | not serious | serious[c] | none | 141 | 80 | - | MD **1.78 higher** (0.13 higher to 3.44 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Alkaline phosphatase (U/L) by intake: Supplementation 16 to 25 mg/d Zn** | | | | | | | | | | | | |
| 1 | RCT | serious[k] | not serious | not serious | serious[l] | none | 30 | 30 | - | MD **12 higher** (11.81 lower to 35.81 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Alkaline phosphatase (U/L) by intake: Supplementation 26 to 50 mg/d Zn** | | | | | | | | | | | | |
| 2 | RCT | very serious[m] | not serious | not serious | serious[l] | none | 151 | 85 | - | MD **2.33 higher** (2.23 lower to 6.89 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Alkaline phosphatase (U/L) by intake: Supplementation 51 to 100 mg/d Zn** | | | | | | | | | | | | |
| 1 | RCT | serious[n] | not serious | not serious | serious[l] | none | 32 | 32 | - | MD **6 higher** (21.65 lower to 33.65 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Alkaline phosphatase (U/L) by supplementation type: Zinc sulphate** | | | | | | | | | | | | |
| 1 | RCT | serious[k] | not serious | not serious | serious[l] | none | 30 | 30 | - | MD **12 higher** (11.81 lower to 35.81 higher) | ⊕⊕◯◯ Low | IMPORTANT |
| **Alkaline phosphatase (U/L) by supplementation type: Zinc gluconate** | | | | | | | | | | | | |
| 2 | RCTs | very serious[m] | not serious | not serious | serious[o] | none | 277 | 150 | - | MD **2.76 higher** (1.11 lower to 6.64 higher) | ⊕◯◯◯ Very low | IMPORTANT |

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| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | ALP | Control | Relative (95% CI) | Absolute (95% CI) | | |
| **Alkaline phosphatase (U/L) by supplementation type: Zinc acetate** | | | | | | | | | | | | |
| 1 | RCT | serious[n] | not serious | not serious | serious[l] | none | 32 | 32 | - | MD **6 higher** (21.65 lower to 33.65 higher) | ⨁⨁◯◯ Low | IMPORTANT |
| **Alkaline phosphatase (U/L) by supplementation type: Depletion** | | | | | | | | | | | | |
| 3 | NRS | very serious[p] | serious[i] | not serious | serious[c] | none | 25 | 25 | - | MD **7.63 higher** (4.02 lower to 19.28 higher) | ⨁◯◯◯ Very low | IMPORTANT |

**CI:** confidence interval; **MD:** mean difference**; RCT**: randomized control trial; **NRS**: non-randomized studies

## Explanations

a. Seven papers included in the analysis, four RCTs and three NRS. RCTs – Four had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias), one had unclear risk of bias and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – Three had high risk of bias in confounding and selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, five had high risk of bias and two had unclear risk of bias.

b. Wide difference in point estimates, considerable heterogeneity, $I^2$ >35%.

c. Small number of events, wide confidence intervals including appreciable benefit and harm.

d. Publication bias suspected because of asymmetrical funnel plot.

e. One NRS included in the analysis - High risk of bias in confounding and selection of participants into the study (selection bias), high risk of bias in deviations from intended interventions (performance bias), and high risk of bias in missing outcome data (attrition bias).

f. Three papers included in the analysis, two RCTs and one NRS. RCTs – Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias), and one unclear risk of bias in the selection of the reported results (selective outcome reporting bias). NRS –High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

g. Three papers included in the analysis, two RCTs and one NRS. RCTs – Two had unclear risk of bias in the randomization process (selection bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

h. Two NRS included in the analysis. NRS – Two had high risk of bias in confounding and selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, two had high risk of bias.

i. Wide difference in point estimates, serious heterogeneity, $I^2$ >75%.

j. Two papers included in the analysis, one RCTs and one NRS. RCTs – Unclear risk of bias in the randomization process (selection bias), and high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS – High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias.

k. One RCT - Unclear risk of bias in the randomization process (selection bias), and unclear risk of bias in the selection of the reported results (selective outcome reporting bias).

l. Wide confidence intervals including appreciable benefit and harm.

m. Two RCTs included in the analysis - Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias), and one high risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, two had high risk of bias.

n. One RCT - Unclear risk of bias in the randomization process (selection bias).

o. Confidence intervals indicative of appreciable benefit and harm.

p. Three NRS – Three had high risk of bias in confounding and selection of participants into the study (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, three had high risk of bias.

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## Grade Evidence table: Other biomarkers

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Other biomarkers | control | Relative (95% CI) | Absolute (95% CI) | | |
| **Serum superoxide dismutase (SOD)** | | | | | | | | | | | | |
| 2 | RCTs | very serious[a] | not serious | not serious | serious[b] | none | 44 | 48 | - | MD **0.42 U/mL higher** (0.71 lower to 1.55 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Erythrocyte superoxide dismutase (SOD)** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[c] | serious[d] | not serious | serious[b] | none | 276 | 149 | - | SMD **0.3 SD higher** (0.26 lower to 0.85 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Fasting glucose: All studies** | | | | | | | | | | | | |
| 5 | RCTs / NRS | very serious[e] | serious[f] | not serious | serious[b] | none | 113 | 120 | - | MD **0.68 mg/dL lower** (4.56 lower to 3.19 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Fasting glucose by dose: Supplementation 16 to 25 mg/d Zn** | | | | | | | | | | | | |
| 1 | NRS | very serious[g] | not serious | not serious | serious[h] | none | 7 | 7 | - | MD **1.4 mg/dL lower** (12.87 lower to 10.07 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Fasting glucose by dose: Supplementation 26 to 50 mg/d Zn** | | | | | | | | | | | | |
| 4 | RCTs | very serious[i] | serious[j] | not serious | serious[b] | none | 106 | 113 | - | MD **0.62 mg/dL lower** (4.98 lower to 3.74 higher) | ⊕◯◯◯ Very low | IMPORTANT |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Other biomarkers | control | Relative (95% CI) | Absolute (95% CI) | | |
| **Fasting insulin: All studies** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[k] | not serious | not serious | serious[b] | none | 53 | 53 | - | MD **2.02 µIU/ml lower** (3.01 lower to 1.02 lower) | ⊕◯◯◯ Very low | CRITICAL |
| **Fasting Insulin by sex: Males** | | | | | | | | | | | | |
| 1 | NRS | very serious[l] | not serious | not serious | serious[h] | none | 7 | 7 | - | MD **2.1 µIU/ml lower** (6.25 lower to 2.05 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Fasting Insulin by sex: Females** | | | | | | | | | | | | |
| 2 | RCTs | very serious[m] | serious[n] | not serious | serious[h] | none | 46 | 46 | - | MD **1.65 µIU/ml lower** (3.63 lower to 0.33 higher) | ⊕◯◯◯ Very low | IMPORTANT |
| **Hair zinc** | | | | | | | | | | | | |
| 4 | RCTs | very serious[o] | serious[j] | not serious | serious[b] | none | 191 | 190 | - | MD **7.52 µg/g higher** (0.94 lower to 15.99 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Nail zinc** | | | | | | | | | | | | |
| 2 | RCTs | very serious[p] | serious[q] | not serious | serious[h] | none | 126 | 102 | - | MD **10.47 µg/g higher** (12.09 lower to 33.03 higher) | ⊕◯◯◯ Very low | CRITICAL |

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Other biomarkers | control | Relative (95% CI) | Absolute (95% CI) | | |
| **Brain derived neurotrophic factor (BDNF)** | | | | | | | | | | | | |
| 2 | RCTs | serious[r] | serious[s] | not serious | serious[b] | none | 49 | 54 | - | MD **2.79 ng/mL higher** (3.23 lower to 8.8 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Insulin-like growth factor 1 (IGF-1)** | | | | | | | | | | | | |
| 2 | RCT / NRS | very serious[t] | serious[u] | not serious | serious[b] | none | 104 | 101 | - | MD **3.15 µg/L higher** (49.6 lower to 55.91 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Interleukin 6 (IL-6)** | | | | | | | | | | | | |
| 2 | RCTs | serious[v] | not serious | not serious | serious[h] | none | 40 | 40 | - | MD **0.64 pg/mL lower** (1.18 lower to 0.1 lower) | ⊕⊕◯◯ Low | CRITICAL |
| **Insulin Resistance (HOMA-IR)** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[w] | serious[x] | not serious | serious[h] | none | 53 | 53 | - | MD **0.08 lower** (0.69 lower to 0.53 higher) | ⊕◯◯◯ Very low | CRITICAL |
| **Total Antioxidant Capacity (TAC)** | | | | | | | | | | | | |
| 3 | RCTs / NRS | very serious[y] | serious[q] | not serious | serious[z] | none | 62 | 65 | - | MD **116.96 µmol/L higher** (25.46 higher to 208.45 higher) | ⊕◯◯◯ Very low | CRITICAL |

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Ceballos-Rasgado, et al.

| Certainty assessment | | | | | | | № of patients | | Effect | | Certainty | Importance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| № of studies | Study design | Risk of bias | Inconsistency | Indirectness | Imprecision | Other considerations | Other biomarkers | control | Relative (95% CI) | Absolute (95% CI) | | |
| **Exchangeable Zinc Pool (EZP)** | | | | | | | | | | | | |
| 2 | RCTs / NRS | serious[ab] | not serious | not serious | serious[h] | none | 59 | 59 | - | MD **14.44 mg higher** (9.44 higher to 19.44 higher) | ⨁⨁◯◯ Low | CRITICAL |

**CI:** confidence interval; **MD:** mean difference; **SMD:** standardized mean difference; **RCT**: randomized control trial; **NRS**: non-randomized studies

## Explanations

a. Two RCTs included in the analysis. Two had unclear risk of bias in randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias), and one had unclear risk of bias in the selection of the reported results (selective outcome reporting bias). Overall, one had high risk of bias and one had unclear risk of bias.

b. Small number of events, wide confidence intervals including appreciable benefit and harm.

c. Three papers included in the analysis, two RCTs and one NRS. RCTs – Two had unclear risk of bias in the randomization process (selection bias), and one had high risk of bias in the selection of the reported results (selective outcome reporting bias). NRS - Unclear risk of bias in confounding and high risk of bias in selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

d. Wide difference in point estimates, $I^2 > 80\%$.

e. Five papers included in the analysis, Four RCTs and one NRS. RCTs – Four had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and two had high risk of bias in missing outcome data (attrition bias). NRS - High risk of bias in confounding and selection of participants into the study (selection bias). Overall, three had high risk of bias and two had unclear risk of bias.

f. Wide difference in point estimates, considerable heterogeneity, $I^2 > 60\%$.

g. One NRS - High risk of bias in in confounding and selection of participants into the study (selection bias).

h. Wide confidence interval including appreciable benefit and harm.

i. Four RCTs included in the analysis. Four had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias) and two had high risk of bias in missing outcome data (attrition bias). Overall, two had high risk of bias and two had unclear risk of bias.

j. Wide difference in point estimates, serious heterogeneity, $I^2 > 70\%$.

k. Three papers included in the analysis, two RCTs and one NRS. RCTs – Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). NRS - High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

l. One NRS - High risk of bias in in confounding and selection of participants into the study (selection bias).

m. Two RCTs included in the analysis. Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias and one had unclear risk of bias.

n. $I^2 > 35\%$.

o. Four RCTs included in the analysis. Three had unclear risk of bias in the randomization process (selection bias), one had unclear risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias and two had unclear risk of bias.

p. Two RCTs included in the analysis. Two had unclear risk of bias in the randomization process (selection bias), one had unclear risk of bias in deviations from intended interventions (performance bias), one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias and one had unclear risk of bias.

q. Wide difference in point estimates, considerable heterogeneity, $I^2 > 80\%$.

r. Two RCTs included in the analysis. One had unclear risk of bias in the randomization process (selection bias).

s. Wide difference in point estimates, serious heterogeneity, $I^2 > 85\%$.

t. Two studies included in the analysis, one RCT and NRS. RCT at low risk. NRS – high risk of bias in confounding and selection of participants into the study (selection bias), high risk of bias in missing outcome data (attrition bias), and high risk of bias in measurement of the outcome (detection bias). Overall, one study at high risk of bias.

u. Wide difference in point estimates, $I^2 > 35\%$.

v. Two RCTs included in the analysis. One had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias.

w. Three papers included in the analysis, two RCTs and one NRS. RCTs – Two had unclear risk of bias in the randomization process (selection bias), one had high risk of bias in deviations from intended interventions (performance bias), and one had high risk of bias in missing outcome data (attrition bias). NRS - High risk of bias in confounding and selection of participants into the study (selection bias). Overall, two had high risk of bias and one had unclear risk of bias.

x. Serious heterogeneity, $I^2 > 75\%$.

y. Three papers included in the analysis, two RCTs and one NRS. RCTs - Two had unclear risk of bias in the randomization process (selection bias). NRS – Unclear risk of bias in confounding and high risk of bias in selection of participants into the study (selection bias). Overall, one had high risk of bias and two had unclear risk of bias. Confidence intervals including appreciable benefit and harm.

z. Confidence intervals including appreciable benefit and harm.

aa. One RCTs included in the analysis. Unclear risk of bias in the randomization process (selection bias).

ab. Two studies included in the analysis, one RCT and one NRS. NRS - High risk of bias in confounding, high risk of bias in deviations from intended interventions (performance bias), high risk of bias in missing outcome data (attrition bias). Overall, one had high risk of bias.