Triangulating evidence for the causal impact of single-intervention zinc supplement on glycaemic control for type 2 diabetes: systematic review and meta-analysis of randomised controlled trial and two-sample Mendelian randomisation

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Abstract

Although previous studies suggested the protective effect of Zn for type 2 diabetes (T2D), the unitary causal effect remains inconclusive. We investigated the causal effect of Zn as a single intervention on glycaemic control for T2D, using a systematic review of randomised controlled trials and two-sample Mendelian randomisation (MR). Four primary outcomes were identified: fasting blood glucose/fasting glucose, HbA1c, homeostatic model assessment for insulin resistance (HOMA-IR) and serum insulin/fasting insulin level. In the systematic review, four databases were searched until June 2021. Studies, in which participants had T2D and intervention did not comprise another co-supplement, were included. Results were synthesised through the random-effects meta-analysis. In the two-sample MR, we used single-nucleotide polymorphisms (SNP) from MR-base, strongly related to Zn supplements, to infer the relationship causally, but not specified T2D. In the systematic review and meta-analysis, fourteen trials were included with overall 897 participants initially. The Zn supplement led to a significant reduction in the post-trial mean of fasting blood glucose (mean difference (MD): −26·52 mg/dl, 95 % CI (−35·13, −17·91)), HbA1c (MD: −0·52 %, 95 % CI: (−0·90, −0·13)) and HOMA-IR (MD: −1·65, 95 % CI (−2·62, −0·68)), compared to the control group. In the two-sample MR, Zn supplement with two SNP reduced the fasting glucose (inverse-variance weighted coefficient: −2·04 mmol/l, 95 % CI (−3·26, −0·83)). From the two methods, Zn supplementation alone may causally improve glycaemic control among T2D patients. The findings are limited by power from the small number of studies and SNP included in the systematic review and two-sample MR analysis, respectively.

Key words: Zn: Type 2 diabetes: Mendelian randomisation: Meta-analysis

Type 2 diabetes (T2D) is a chronic disease that causes a patient's body not to respond normally to insulin called insulin resistance. Impaired insulin sensitivity reduces both the absorption and reserve of insulin in organs. At an early stage, the patient's serum insulin levels will increase to compensate for the increased insulin resistance. However, serum insulin will eventually decrease and blood sugar levels rise[1,2]. Diabetes symptoms include polydipsia, fatigue and unintended weight loss and cause lower self-evaluation of physical and mental health[3]. Globally, the age-standardised incidence rate increased by 3·23 % to 279·13 per one million from 2007 to 2017[4]. It brings multiple challenges to medicine, primary care and the economy. Four-fifths of diabetes patients currently live in low- and middle-income countries,

and diabetes increase their susceptibility to other infections such as hepatitis B[5]. In India, nearly half of patients with T2D did not receive a proper diagnosis and are at risk of hidden complications as the disease progresses[6]. Moreover, patients faced substantial cost-of-illness and lower employment chances, while government health expenditure on T2D is projected to decline[7,8]. It is therefore critical to find a cost-effective and accessible intervention to address the threat of T2D. Zn, as a usual commercial supplement, could be a good candidate solution.

Zn supplementation was demonstrated to have a protective effect on insulin and metabolism in the hyperglycaemic environment in animal models[9–11]. The Zn ion plays an essential role

with insulin in the pancreatic β cell, activating multiple cell signalling cascades[12]. Zn ions coordinate six insulin monomers (hexamerisation), which enhance the stability of insulin and the storage capacity of the insulin-secreting vesicles[13–15]. Moreover, Zn is known for its antioxidative property as a cofactor of the important antioxidative enzyme, which may reduce lipid peroxidation and development of insulin resistance in diabetes mellitus[16–18].

Randomised controlled trials (RCT) have evaluated the impact of Zn or co-supplements on various types and progression of diabetes[19,20]. One meta-analysis, published in 2019, found that Zn supplements in both single factor and co-supplements significantly reduced glycaemic indicators such as fasting glucose[21]. A 2021 meta-analysis suggested that low-dose and long-duration single Zn supplements had a beneficial impact on many T2D and cardiovascular disease risk factors[22]. The multi–nutrient intervention in the previous review included the interactions between nutrients, so it may not provide a valid estimate of Zn 's effect as a single supplement. For example, Fe and Ca supplements may interfere with the repletion of Zn and inhibit Zn's bioaccessibility, and the interaction mechanism within more micronutrients remained complicated[23,24]. Neither study was limited to a specific type of diabetes and even included healthy participants at high risk. This could mask the real effect of the single Zn supplement on prevalent T2D patients.

Mendelian randomisation (MR) is another study design that could help us to evaluate the causality between single Zn supplements and diabetes. MR is regarded as a 'natural experiment', which leverages the random inheritance of genetic variants to approximate the random allocation of the participants to a modifiable exposure, which the variants are robustly associated with[25–28]. Because genes are inherited at birth, MR estimates are interpreted as the lifetime effect of the exposure, while RCT studies can only provide the effects over shorter periods of time. Single-nucleotide polymorphisms (SNP) are a one-row change in the DNA double helix which occurs in at least 5 % of the population, which are the most common type of genetic variant. The associations between SNP and phenotypes are generally detected through genome-wide association studies (GWAS). These studies involve a hypothesis-free test of association between every measured SNP and the phenotype. To prevent false positives, stringent multiple testing criteria are applied when deciding if an SNP is 'genome-wide significant'. By measuring the whole genome, it is, therefore, possible to characterise the association of SNP with exposure and outcomes, such as metabolic response to a specific diet[29–31]. Nevertheless, because GWAS are hypothesis-free, they do not provide insight into the mechanism linking the SNP with an associated phenotype-like Zn supplementation. It is, however, unlikely that any associated SNP would be a 'gene for zinc supplementation'. Instead, they would likely be SNP that influence risk factors for taking Zn supplementation (such as a generic propensity to health-seeking behavior), or SNP that happen to have a different prevalence in subgroups who are more likely to take the supplements (residual population structure).

MR has been used to obtain an unbiased assessment of nutritional status to the outcome of interest. Researchers notably demonstrated the association of long-term testosterone exposure with health outcomes by MR[32]. Collaborating with RCT, MR could help to strengthen the causal estimation, particularly in cumulating exposure inside the body[33], which may provide suggestions for health professionals to give patients accessible supplements for diabetes management, especially under the source-limited condition. In this study, we aimed to systematically assess the unmasked effect and possible causal inference about the association between single Zn supplements and glycaemic control among T2D patients, using a systematic review of RCT and two-sample MR.

Method

Systematic review

Protocol and ethnics. This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline[34]. This study received ethics approval from the London School of Hygiene and Tropical Medicine (LSHTM) MSc Research Ethics Committee (reference: 25 896).

Eligibility criteria. The study eligibility criteria were specified using Population, Intervention, Comparison, Outcomes and Study (PICOS) frame[34].

Inclusion criteria.
- Study population: Any human participants with T2D. There was no restriction on the demographic characteristics.
- Intervention: Zn administration as the single supplement intervention.
- Comparison: Use of placebo.
- Outcome: Primary outcomes included fasting blood glucose, HbA1c, homeostatic model assessment for insulin resistance (HOMA-IR) and serum insulin level. Secondary outcomes included any other quantitative outcomes related to diabetes control.
- Study design: Any RCT evaluating the association between Zn intervention and glycaemic control.

Exclusion criteria. • Full text was not available or accessible.
- Co-supplement with other nutrition would not be included to concentrate on the Zn-only effect.

Information sources and search strategy. PubMed, CINAHL Plus, EMBASE and Web of Science were searched from the establishment of each database (in 1996, 1937, 1961 and 1997, respectively) to June 2021. We also checked the references and citations of all included studies for eligibility criteria. The search strategy was developed by and modified from the previous systematic review[21]. The terms were structured according to each database's guidelines and searched by titles and abstracts. The detail is presented in supplementary method 1.

Selection process. The abstracts and titles were screened by the eligibility criteria. The first unblinded reviewer screened all the citations and the second one checked a random 10 % sample

of all citations due to the limitation of human resources. The percentage agreement and the Gwet AC (agreement change adjusted) between the two reviewers were calculated to test the interrater reliability[35].

Data extraction and item. The standard form for intervention reviews for RCT developed by the Cochrane Collaboration was used to exact the data[36]. The extraction process was conducted twice to minimise bias or entry error. The specific data items are shown below:

- Eligibility criteria: inclusion and exclusion criteria
- Study design (parallel or crossover), assignment of each arm and analysis plan (intention-to-treat or per-protocol)
- Participants: the total number of randomised and analysed participants, numbers in each arm (pre-trial and post-trial), geographic information and demographic characteristics.
- Intervention and control arms: formulation and dosage of Zn, administration method, description of the control arm (placebo) and study duration.
- Results: the measure of effect (means, mean difference or change score), standard error of effect measure, statistical significance and any other results such as OR.
- Information about risk-of-bias: any information causing bias, such as missing participants.

Effect measure. The primary measure was the post-trial mean difference (MD) with standard error (SE) for each outcome. The change scores are the change of mean from pre-trial to post-trial in each arm so that the difference of change scores between Zn intervention and control was our secondary effect measure. We either converted units or used the standardised mean difference (SMD) to harmonise the measurement scales[37].

Synthesis methods. The meta-analyses were conducted to synthesise the results. The subgroup analyses were stratified by studies that clearly stated the participants were T2D patients and that did not specify the participant's diabetes type.

The Cochran-Q χ^2 test and the I^2 index were used to assess the statistical heterogeneity. We also examined previous systematic reviews for their findings on heterogeneity. If there was no statistically significant heterogeneity ($P > 0.05$ and $I^2 < 40\%$) or evidence about existing heterogeneity from previous studies, a pooled effect would have been calculated with a fixed-effects model, which assumed that the observed differences among study results are due solely to chance[37]. If there was significant heterogeneity, the summary estimate and confidence/prediction interval would come from the random-effects model, which assumed that the different studies are estimated differently, then providing the average intervention effect[37].

If the study included more than two intervention arms, we reviewed only interventions that met the eligibility criteria or summarised at the arm level. We only interpreted studies that were judged as low risk of overall bias in the main result section to avoid biases.

Reporting bias assessment. If there were five or more studies in the meta-analysis, we assessed publication bias by graphical methods (funnel plots, which indicate the potential presence of reporting biases)[38]. In the plots, the x-axis represents the effect estimate, and the y-axis represents the standard error of the effect estimate. If there was publication bias, it would lead to an asymmetrical appearance of the plot[39]. Furthermore, the Egger test was used for reporting bias to evaluate the small study effect[40].

Additional analysis. If the heterogeneity was significant in the meta-analysis, the linear random-effect meta-regression was used to explore the trial-level covariates that contributed to the heterogeneity[41]. The candidate covariates were Zn dose, trial duration and whether the trial specified the participant's diabetes type (T2D, not specified types). The first two were treated as continuous variables and the third was a binary variable.

We illustrated the effect of interest if only one study reported any secondary outcomes. If more than one study reported a type of secondary outcome, a meta-analysis was conducted regardless of the risk-of-bias. We also performed a sensitivity analysis which included all trials into meta-analysis regardless of risk assessment for comparing the results to evaluate the impact of bias.

Two-sample Mendelian randomisation

This two-sample MR study followed STROBE-MR Guidelines[42].

Study design and data sources. Sample sizes and GWAS data including both exposure and outcome were utilised from the MR-base, which is a repository of GWAS summary statistics[43]. We included all 'zinc supplement' SNP (Open GWAS ID: ukb-b-10567, ukb-b-13891, ukb-a-496) that meet the p-value threshold of $<5 \times 10^{-8}$ from the MR-base GWAS catalog. We also extracted the summary data (β and SE) for the diabetes outcomes: fasting blood glucose (Open GWAS ID: ieu-b-114, GCST000568, ebi-a-GCST007858, ebi-a-GCST005186, ieu-b-113), HbA1c (Open GWAS ID: ieu-b-103, ieu-b-104), HOMA-IR (Open GWAS ID: ieu-b-118, ebi-a-GCST005179) and fasting insulin (Open GWAS ID: ebi-a-GCST005185, ebi-a-GCST000571, ebi-a-GCST007857, ieu-b-115, ieu-b-116). Description of the methods for these GWAS can be found in reference[44–46].

We performed clumping using the genetic distance of 10 000 kilobases and an linkage disequilibrium R^2 of 0·001 from the 1000 genomes European reference sample. When SNP were not measured in the outcome data set, we used proxy SNP with a minimum linkage disequilibrium R^2 of 0·8[47]. Palindromic SNP were imputed if they had a minor allele frequency at <0.3[48,49]. We allowed MR-base to automatically harmonise the exposure and outcome data and attempt to align palindromic SNP based on their minor allele frequency.

Assumptions and assessment. There are three routine assumptions and two additional assumptions in two-sample MR.

1. Relevance: The genetic variants are associated with the exposure of interest.

✓ This assumption was assessed via F statistic for the gene-exposure relationship.

2. Independence: The genetic variants share no unmeasured cause with the outcome.

✓ This assumption was by ensuring that the GWAS have adequately controlled for plausible confounders of the gene-outcome association by adjusting for at least ten principal components or using a linear mixed model.

3. Exclusion restriction: The genetic variants do not affect the outcome except through their potential effect on the exposure of interest.

✓ Pleiotropy can violate this assumption when genes influence two or more traits. Several secondary analyses were used to detect and adjust the pleiotropy such as MR-Egger regression, weighted median and weighted mode analysis.

4. The samples for exposure and outcome assessment are independent.

✓ We excluded the outcome summary data which had the same consortium as the exposure data to meet this assumption.

5. The samples for exposure and outcome assessment are from the same population.

✓ We chose the same outcome population category such as European with the exposure population, ensuring the fifth assumption.

Statistical methods. We used the 'TwoSampleMR' package in the R environment (version 4.1.0) to conduct the two-sample MR analyses[43]. This package supported the harmonisation process to ensure that both SNP were coded from the same strand when SNP were palindromic[48]. We used the inverse variance weighting estimator to evaluate the causal inference. It meta-analyses the Wald ratios which is the ratio of the SNP outcome association to SNP exposure association. Due to the difference in MR-base, two outcomes were slightly changed compared with the systematic review: fasting glucose and fasting insulin.

Cochran Q statistics can be used to assess evidence of heterogeneity in consideration of pleiotropy[50]. The statistically significant level was at the $P < 0.05$. Steiger filtering checked the direction of causation between the exposure and outcome for each SNP[51].

We, therefore, used the exposure of Ca supplement and outcome of hair colour as the negative controls to detect the potential residual bias due to nutrients supplementation and population structure, respectively. These were chosen because Ca supplementation is a type of nutritional supplement which is not thought to impact diabetes, and hair colour is known to vary with population structure within the UK but is unlay to have any true causal association with Zn supplementation.

Results

Systematic review

Study selection. A total of fifteen studies, reporting on fourteen trials, were identified for inclusion in the review (Fig. 1). One trial generated two studies (Parham *et al.*[62] and Heidarian *et al.*[63]). From the four databases, 1557 citations were found, and three studies were identified by reference reading. 1531 citations were removed because of duplication or unfulfilling inclusion criteria with title or abstract. After the full-text screening of twenty-nine studies, fourteen studies were excluded because they did not meet the criteria. There were 11, 12, 7 and 8 studies measuring fasting blood glucose, HbA1c, HOMA-IR and serum insulin levels, respectively.

The percentage agreement was 96·32 % and Gwet AC was 0·9619 between two reviewers, with strong evidence of significance ($P < 0.05$), which indicated a very good inter-reviewer reliability for the selection process. No unpublished relevant studies were obtained.

Study characteristics. Thirteen trials used a parallel design and one used a cross-over design (Parham *et al.*[62] and Heidarian *et al.*[63]). Details of each trial are presented in Table 1. A total of 897 participants were randomly allocated at initial, and all trials had a relatively small sample size. The date of publication ranged from 2003 to 2021. Most trials claimed that there was no significant imbalance ($n=7$) in age and sex, or they used matched methods ($n=2$). One trial had adolescent participants, while the other's average ages were above 45-year-old. Four trials did not specify the type of diabetes in the inclusion criteria and patients had complications: diabetic foot ulcer[59], diabetic haemodialysis with Zn deficiency[56], diabetic retinopathy[60] and β-thalassemia major complicated[58].

The duration of Zn supplementation ranged from 1·5 to 12 months, with a mean and median duration of 3·53 months and 3 months. Three trials contained basic supplements care besides the Zn administration and placebo: oral hypoglycaemic agent (in Burki *et al.*[54] and Khan *et al.*[57]) and multivitamin/mineral (in Gunasekara *et al.*[55]). Gunasekara *et al.*[55] had three arms and we only extracted the information of the Zn+ multivitamin/mineral (intervention) and multivitamin/mineral group (control). Each dose of Zn gluconate and Zn sulfate was multiplied by 0·143 or 0·227 to obtain the appropriate dose of elemental Zn[22]. The mean dose of elemental Zn included in these interventions was 25·83 mg/d (range: 4·29–149·82 mg/d; median: 9·25 mg/d).

Results of individual studies. Nine trials measured post-trial means with SE of fasting blood glucose as outcomes. Seven of the nine trials were at low risk of bias. Eight trials including the cross-over trial measured post-trial HbA1c as an outcome. Six of the eight trials were at low risk of bias. Six trials with low risk-of-bias had eligible measured post-trial HOMA-IR scores. Six trials with low risk-of-bias had eligible measured post-trial serum insulin as an outcome. We standardised the measurement of serum insulin level on a single scale through Hedges' g score and presented the SMD.

The detail of the risk of bias assessment for each study is presented in Supplementary result 1, Supplementary Fig. 1 and Supplementary Table 1.

Synthesis of results. The random-effects model was used for meta-analysis for all outcomes, because of the I^2 (>40 %), the
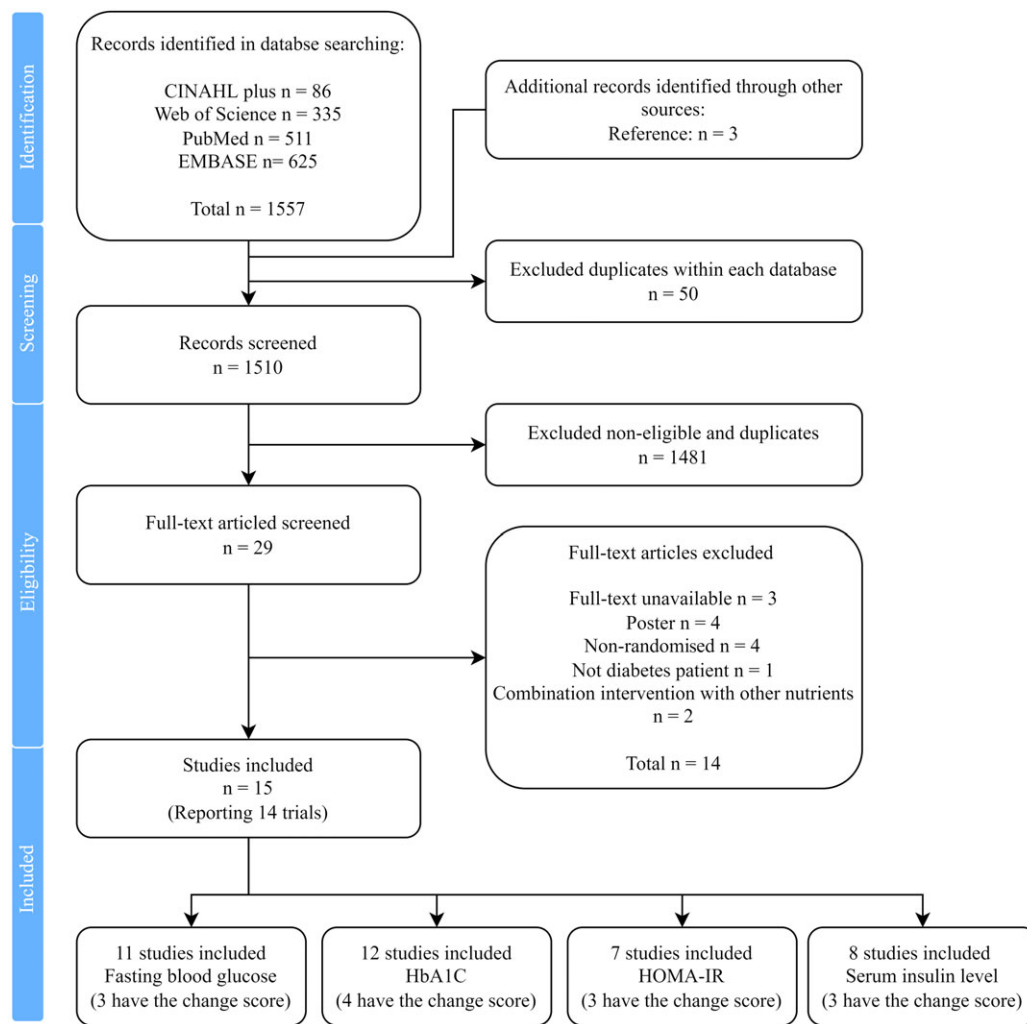
Fig. 1. PRISMA flow diagram of information through the different phases of the systematic review.

Cochran-Q test ($P < 0.05$) in most outcomes and the findings of previous systematic reviews[22]. The meta-analyses including the only low risk-of-bias trials are presented in Fig. 2.

Fasting blood glucose. Among the T2D patients, the pooled MD was -27.68 mg/dl (95 % CI (-37.26, -18.11)), comparing Zn intervention to the control arm (Fig. 2(a)). Among the participants without specified diabetes type, the pooled MD was -28.17 mg/dl (95 % CI (-43.88, -12.45)). Both stratified analyses showed strong evidence of effect and the two types of trials were not significantly different (Test of group difference $P = 0.96$). The overall pooled MD showed that the Zn intervention had a significantly lower level of fasting blood glucose at the end of the trial, compared with the control arm (MD: -26.52, 95 % CI (-35.13, -17.91)). Regardless of the Risk-of-bias (ROB) of the trials, the overall pooled MD was -26.87 mg/dl (95 % CI (-35.74, -18.00)) (online Supplementary Fig. 2(a)).

HbA1C. T2D patients have a decrease (MD: -0.48, 95 % CI (-1.06, 0.11)) in HbA1c, but this difference was not significant

between arms (Fig. 2(b)). Among the participants without specified type, the pooled MD was -0.57 % (95 % CI (-0.96, -0.17)) in HbA1c, compared to Zn intervention and control arm. There was no significant difference between those two types of trials (Test of group difference $P = 0.80$). Overall, there was a significant reduction in post-trial HbA1c percentage between Zn intervention and control at the end of the trial (MD: -0.52, 95 % CI (-0.90, -0.13)). The overall pooled MD that also included all trials regardless of the ROB was -0.64 % (95 % CI (-1.05, -0.22)) (online Supplementary Fig. 2(b)).

Homeostatic model assessment for insulin resistance. The pooled MD was -0.49 (95 % CI (-1.26, 0.28)) in T2D patients and -2.23 (95 % CI (-3.05, -1.42)) among patients with unspecified type in the post-trial HOMA-IR significantly (Fig. 2(c)). There was a significant difference detected between those two types of trials (Test of group difference $P < 0.01$). The pooled result shows that the Zn intervention arm had a 1.65 lower (95 % CI (-2.62, -0.68)) HOMA-IR score than the control arm with strong evidence.

Table 1. Summary of included studies evaluating the effect of Zn intervention in the systematic review

Study	RCTdesign	Country	Randomisation participants,n control/intervention*	Duration, month	Sex,male/female	Age (Mean), year†	Age (Standard deviation), year	Formulation/elemental Zn dose,mg/d	Health status
Afkhami and Ardekani, 2008[52]	Parallel	Iran	40 (total)	1·5	16/24	52·67	8·60	Zn sulfate/149·82	Type 2 diabetes
Asghari, 2019[53]	Parallel	Iran	30/30	3	32/28	45·5 v. 46·2	5·4 v. 5·3	Zn gluconate/4·29	Type 2 diabetes
Burki, 2017[54]	Parallel	Pakistan	46/54	3	NA	NA		Zn sulphate/4·54§	Type 2 diabetes
Gunasekara, 201[55]‡	Parallel	Sri Lanka	31/29	4	23/27	51·2 v. 54·1	6·0 v. 6·0	Elemental Zn/22‖	Type 2 diabetes
Hosseini, 2021[56]	Parallel	Iran	25/21	2	22/24	54·1 v. 55·6	5·4 v. 7·3	Elemental Zn/50	Diabetic haemodialysis patients with Zn deficiency
Khan, 2013[57]	Parallel	India	27/27	3	NA	56·0 v. 56·3	6·86 v. 6·6	Zn sulphate/11·35	Type 2 diabetes
Matter, 2020[58]	Parallel	Egypt	40/40	3	38/42	16·1 v. 16·4	1·4 v. 1·4	Zn gluconate/7·72	Diabetes with β-thalassemia major complicated
Momen-Heravi, 2017[59]	Parallel	Iran	30/30	3	42/18	60·0 v. 58·3	10·0 v. 8·6	elemental Zn/50	Diabetes with diabetic foot ulcer
Naghizadeh, 2018[60]	Parallel	Iran	22/23	3	20/25	58·0 v. 57·4	5·9 v. 5·8	Zn gluconate/4·29	Patients with diabetic retinopathy
Nazem, 2019[61]	Parallel	Iran	35/35	2	38/32	54·3 v. 53·3	7·2 v. 7·4	Zn gluconate/7·15	Type 2 diabetes
Parham, 2008[62], Heidarian, 2009[63]	Cross-over	Iran	42 (total)	2·5	24/18	52·0 54·5	9·3 v. 9·2	elemental Zn /30	Type 2 diabetes
Pérez, 2018[64]	Parallel	Chile	15/13	12	13/15	30–65		elemental Zn /6·81	Type 2 diabetes with further selection criteria
Roussel, 2003[65]	Parallel	Tunis	29/27	6	NA	55·5 v. 51·5	1·4 v. 1·6	Zn gluconate/4·29	Type 2 diabetes
Witwit, 2021[66]	Parallel	Iraq	25/25	1·5	NA	25–60		Zn sulphate/11·35	Type 2 diabetes

RCT, randomised controlled trial.

* smaller size of participants due to lost during the trial

† Values are mean (control, intervention) or inclusion range.

‡ It was a three-arm study, and we only extracted Zn and its control arm.

§ Oral hypoglycaemic arm and Zn plus oral hypoglycaemic arm.

‖ Multivitamin/mineral arm and Zn plus multivitamin/mineral arm.
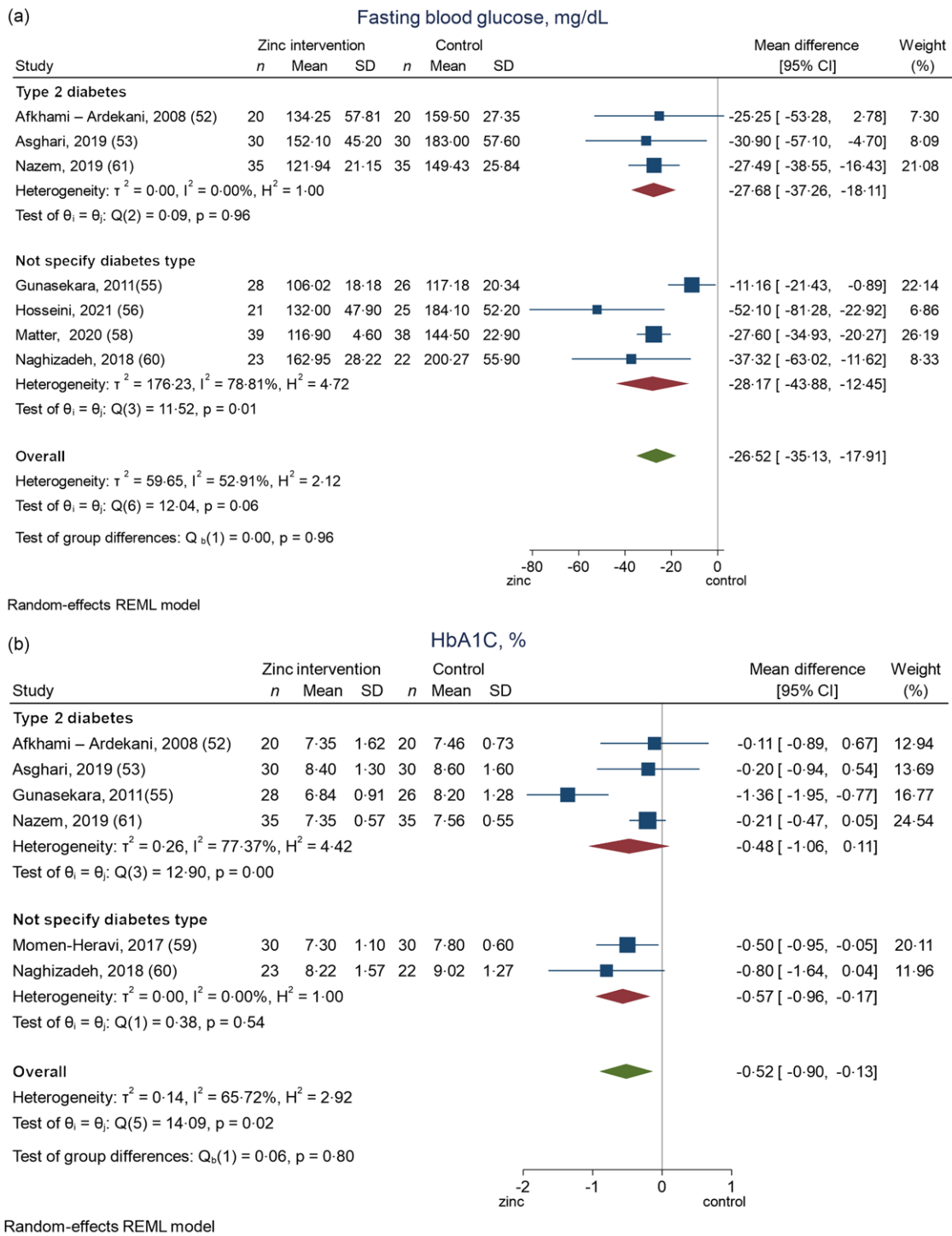
Fig. 2. Forest plots summarising the MD or SMD of post-trial fasting blood glucose (a), HbA1c (b), HOMA-IR (c) and serum insulin level (d) between Zn intervention and control arms for low risk-of-bias trials. MD, mean difference; SMD, standardised mean difference; HOMA-IR, homeostatic model assessment for insulin resistance.

Serum insulin level. For serum insulin level (Fig. 2(d)), the SMD was 0·25 (95 % CI (−0·04, 0·54)) in T2D patients and −0·53 (95 % CI (−1·11, 0·05)) among the participants without specified diabetes type, with only weak evidence. Overall, the pooled result shows that there was no significant SMD in serum insulin level between Zn intervention and control arms at the end of the trial (SMD: −0·12, 95 % CI (−0·55, 0·30)).

The results of the change scores are presented in Fig. 3, but we did not observe any significant results, except with the outcome of HbA1C, which may be because fewer trials reported the measure of change score. The detail of the certainty assessment is presented in Supplementary result 2 and Supplementary Table 2. We did not contact corresponding authors to obtain outcome data if they did not report post-trial means or change scores.

(c)

HOMA-IR

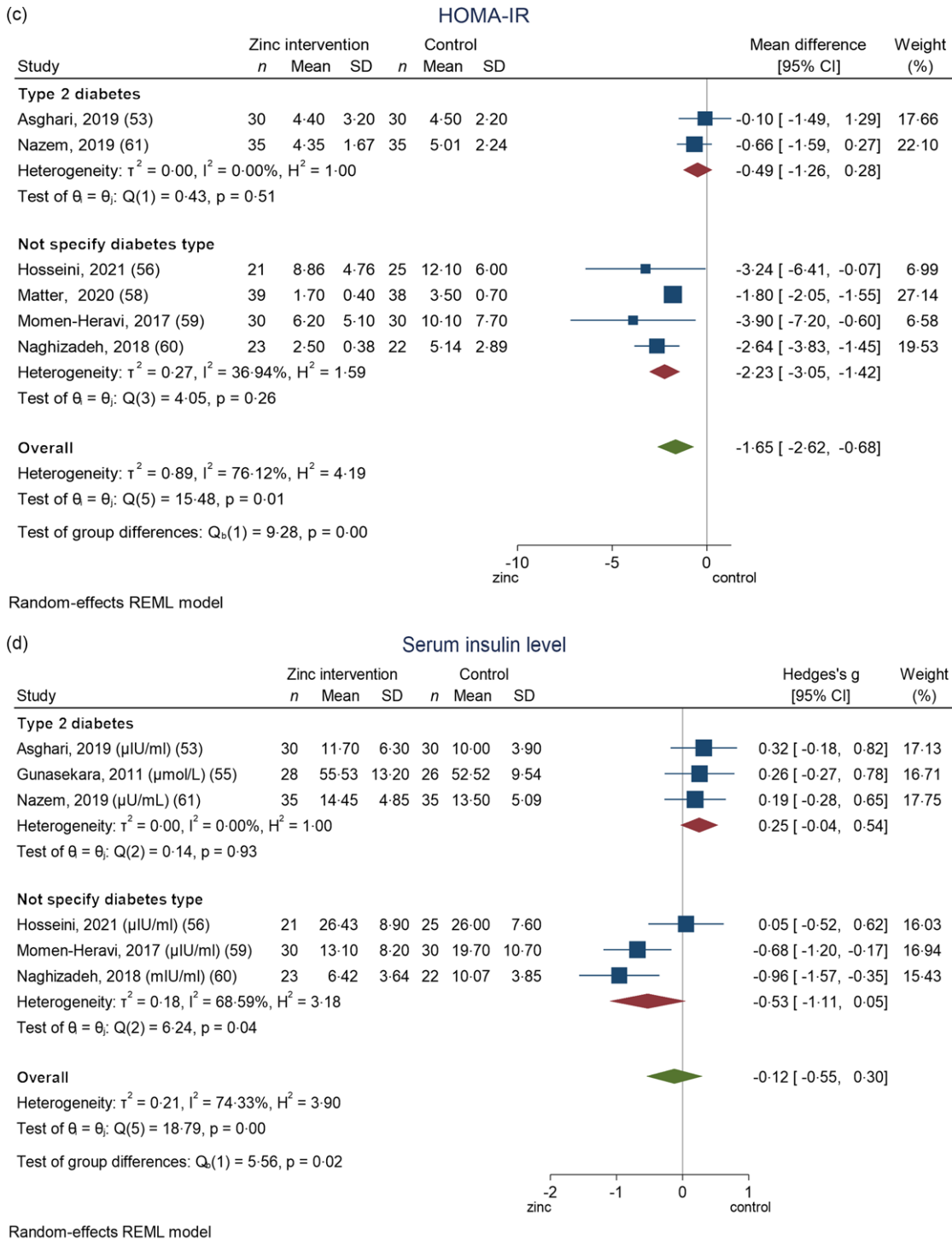| Study	Zinc intervention n	Mean	SD	Control n	Mean	SD	Mean difference [95% CI]	Weight (%)
Type 2 diabetes								
Asghari, 2019 (53)	30	4·40	3·20	30	4·50	2·20	-0·10 [-1·49, 1·29]	17·66
Nazem, 2019 (61)	35	4·35	1·67	35	5·01	2·24	-0·66 [-1·59, 0·27]	22·10
Heterogeneity: $\tau^2 = 0.00$, $I^2 = 0.00\%$, $H^2 = 1.00$							-0·49 [-1·26, 0·28]	
Test of $\theta_i = \theta_j$: Q(1) = 0·43, p = 0·51								
Not specify diabetes type								
Hosseini, 2021 (56)	21	8·86	4·76	25	12·10	6·00	-3·24 [-6·41, -0·07]	6·99
Matter, 2020 (58)	39	1·70	0·40	38	3·50	0·70	-1·80 [-2·05, -1·55]	27·14
Momen-Heravi, 2017 (59)	30	6·20	5·10	30	10·10	7·70	-3·90 [-7·20, -0·60]	6·58
Naghizadeh, 2018 (60)	23	2·50	0·38	22	5·14	2·89	-2·64 [-3·83, -1·45]	19·53
Heterogeneity: $\tau^2 = 0.27$, $I^2 = 36.94\%$, $H^2 = 1.59$							-2·23 [-3·05, -1·42]	
Test of $\theta_i = \theta_j$: Q(3) = 4·05, p = 0·26								
Overall							-1·65 [-2·62, -0·68]	
Heterogeneity: $\tau^2 = 0.89$, $I^2 = 76.12\%$, $H^2 = 4.19$								
Test of $\theta_i = \theta_j$: Q(5) = 15·48, p = 0·01								
Test of group differences: $Q_b(1) = 9.28$, p = 0·00								

-10 (zinc) -5 0 (control)

Random-effects REML model

(d)

Serum insulin level

Study	Zinc intervention n	Mean	SD	Control n	Mean	SD	Hedges's g [95% CI]	Weight (%)
Type 2 diabetes								
Asghari, 2019 (μIU/ml) (53)	30	11·70	6·30	30	10·00	3·90	0·32 [-0·18, 0·82]	17·13
Gunasekara, 2011 (μmol/L) (55)	28	55·53	13·20	26	52·52	9·54	0·26 [-0·27, 0·78]	16·71
Nazem, 2019 (μU/mL) (61)	35	14·45	4·85	35	13·50	5·09	0·19 [-0·28, 0·65]	17·75
Heterogeneity: $\tau^2 = 0.00$, $I^2 = 0.00\%$, $H^2 = 1.00$							0·25 [-0·04, 0·54]	
Test of $\theta_i = \theta_j$: Q(2) = 0·14, p = 0·93								
Not specify diabetes type								
Hosseini, 2021 (μIU/ml) (56)	21	26·43	8·90	25	26·00	7·60	0·05 [-0·52, 0·62]	16·03
Momen-Heravi, 2017 (μIU/ml) (59)	30	13·10	8·20	30	19·70	10·70	-0·68 [-1·20, -0·17]	16·94
Naghizadeh, 2018 (mIU/ml) (60)	23	6·42	3·64	22	10·07	3·85	-0·96 [-1·57, -0·35]	15·43
Heterogeneity: $\tau^2 = 0.18$, $I^2 = 68.59\%$, $H^2 = 3.18$							-0·53 [-1·11, 0·05]	
Test of $\theta_i = \theta_j$: Q(2) = 6·24, p = 0·04								
Overall							-0·12 [-0·55, 0·30]	
Heterogeneity: $\tau^2 = 0.21$, $I^2 = 74.33\%$, $H^2 = 3.90$								
Test of $\theta_i = \theta_j$: Q(5) = 18·79, p = 0·00								
Test of group differences: $Q_b(1) = 5.56$, p = 0·02								

-2 (zinc) -1 0 1 (control)

Random-effects REML model

Fig. 2. (Continued)

Some secondary outcomes were found after full-text reading, and their method and result in *post hoc* analysis are presented in Supplementary Result 3 and Supplementary Fig. 3.

Reporting bias assessment. We observed a slight asymmetry in the funnel plot of the fasting blood glucose in Fig. 4(a). One study fell out of the 95 % CI. The *P*-value of the Egger test for small-study effects was 0·1649, which meant there was no strong reporting bias.

Visual assessment of the funnel plots of HbA1c (Fig. 4(b)), HOMA-IR (Fig. 4(c)) and serum insulin level (Fig. 4(d)) suggested that there was no asymmetry. Further, the *P*-value of the Egger test for small-study effects was 0·7341, 0·2697 and 0·1514 for HbA1c, HOMA-IR and serum insulin level,

(a)
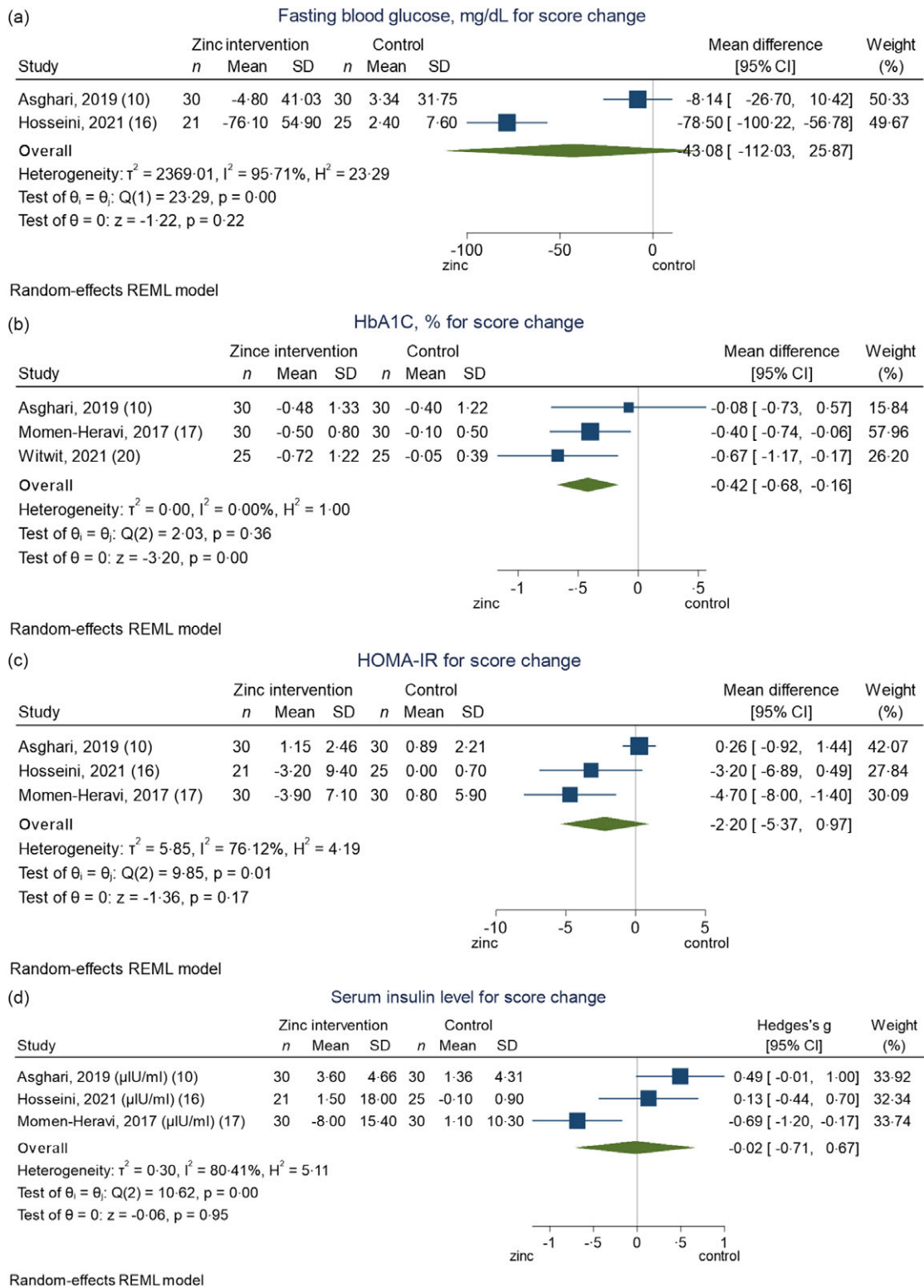
Fig. 3. Forest plots summarising the MD or SMD of change scores among fasting blood glucose (a), HbA1c (b), HOMA-IR (c) and serum insulin level (d) between Zn intervention and control arms for low risk-of-bias trials. MD, mean difference; SMD, standardised mean difference; HOMA-IR, homeostatic model assessment for insulin resistance.
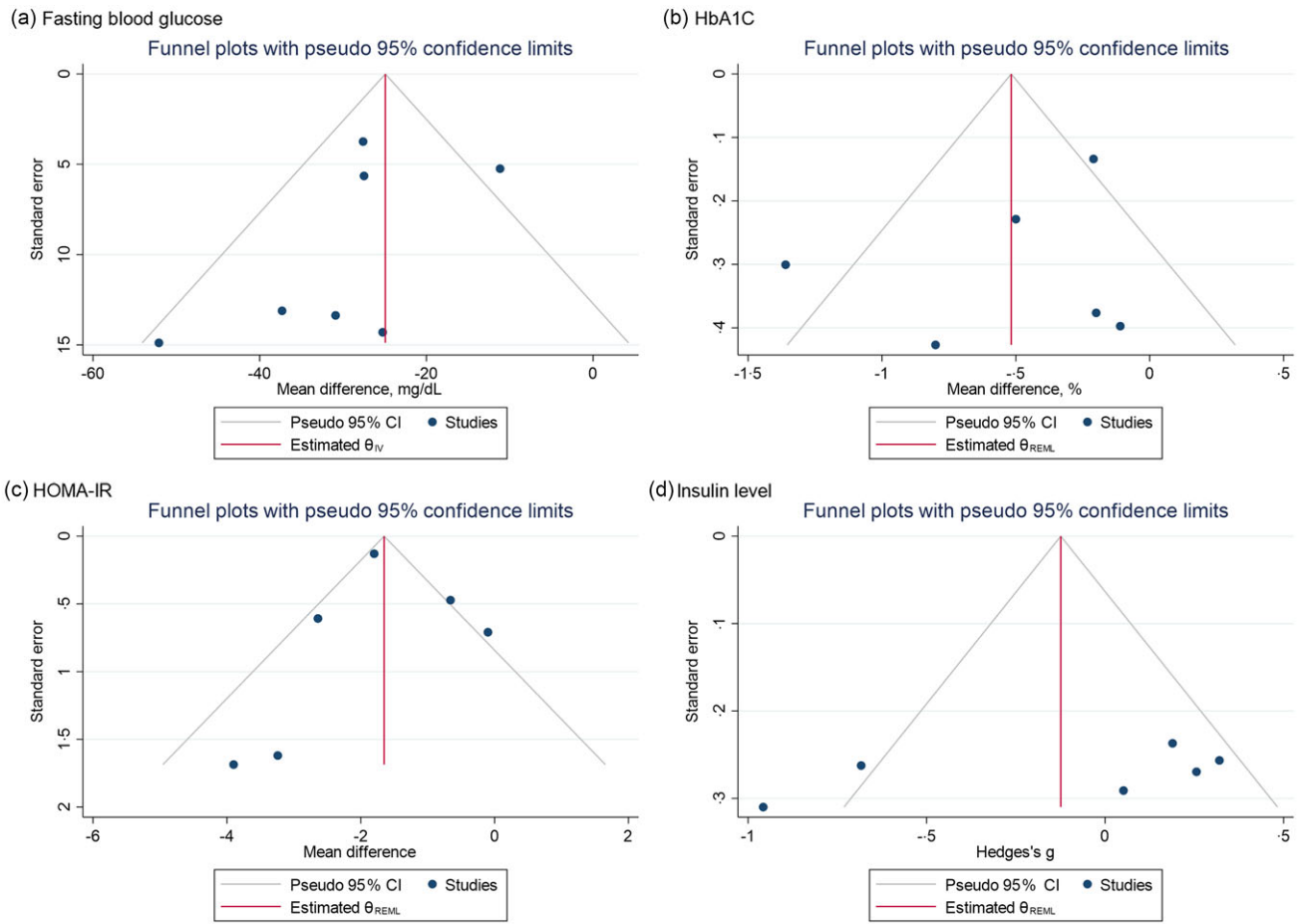
Fig. 4. Funnel plots with pseudo 95 % CI demonstrating the MD or SMD of post-trial fasting blood glucose (a), HbA1c (b), HOMA-IR (C) and serum insulin level (d) for each low risk-of-bias trials against their corresponding SEs. MD, mean difference; SMD, standardised mean difference; HOMA-IR, homeostatic model assessment for insulin resistance.

respectively, which fail to reject the null hypothesis of no asymmetry and suggested no significant reporting bias exist.

Additional analysis: meta-regression. Second, through meta-regression (regardless of the ROB), we found evidence to support a linear association of fasting blood glucose (regression coefficient: 8·42, 95 % CI (0·52, 16·33)) and HbA1c (regression coefficient: −0·46, 95 % CI (−0·73, −0·19)) with trial duration, which was the significant explanatory variable on heterogeneity. Whether specified diabetes types significantly explained the heterogeneity for the outcome of HOMA-IR (regression coefficient: −1·68, 95 % CI (−2·84, −0·51)) and serum insulin level (regression coefficient: −0·78, 95 % CI (−1·31, −0·25)) in the meta-analyses. The regression coefficient reflected that every level change of explanatory variables would lead to the change of effect size with each outcome's unit. We shall be cautious about the results because none of the outcomes had more than ten trials. These results are presented in Table 2.

Two-sample Mendelian randomisation

Description of exposure single-nucleotide polymorphism summary. Three SNP were robustly associated with Zn supplement: rs6756297, rs4861163 and rs10822145, and their GWAS all were conducted from the UK biobank, presented in Table 3. The sample size for the GWAS was 461 384 participants.

Description of outcome single-nucleotide polymorphism summary. The summary statistics of each outcome with each SNP were reported in Table 4. Since the exposure data was from the UK biobank, we chose the MAGIC consortium with the European population for each outcome.

Results of two-sample Mendelian randomisation. The results including the heterogeneity test are presented in Fig. 5. Zn supplement with two SNP led to a significat decrease in fasting glucose (coefficient: −2·04, 95 % CI (−3·26, −0·83)). The unit of fasting glucose was mmol/l[67] (−2·04 mmol/l = −37·09 mg/dl). While there was no strong evidence supporting the relationship of Zn supplements with HbA1c, HOMA-IR and insulin level (*P* > 0·05). The *P*-value for heterogeneity of fasting glucose, HbA1c, HOMA-IR and insulin level were 0·29, 0·66, 0·37 and 0·47. Neither of these results had significant heterogeneity, which suggested no pleiotropy with the null hypothesis.

Table 2. Meta-regression of potential explanatory variables on heterogeneity in the meta-analysis (Mean values, coefficient values and standard errors)

		Explanatory factor								
		Dose of Zn intervention			Duration of trial			Diabetes type specified		
Outcome	n studies	Mean, mg/d	Regression coefficient	95 % CI	Mean, month	Regression coefficient	95 % CI	n studies that specified T2D	Regression coefficient	95 % CI
Fasting blood glucose	7	34·75	−0·01	−0·24, 0·23	2·64	8·42	0·52, 16·33*	3	1·31	−18·43, 21·05
HbA1c	6	39·59	0·00	−0·01, 0·01	2·75	−0·46	0·73, −0·19*	4	−0·13	−1·06, 0·77
HOMA-IR	6	20·24	−0·05	−0·11, 0·01	2·67	−0·46	−2·85, 1·93	2	−1·68	2·84, −0·51*
Serum insulin level	6	22·96	−0·00	−0·03, 0·02	2·83	−0·04	−0·73, 0·65	3	−0·78	−1·31, −0·25*

HOMA-IR, homeostatic model assessment for insulin resistance.
* $P < 0.05$.

Table 3. Description of the Zn supplement-SNP association in the two-sample MR (Coefficient values and standard errors)

	Exposure: Zn supplement				
SNP	Sample size	β	SE	Population	Consortium
rs4861163	461 384	0·006	0·001	European	UK Biobank
rs10822145	461 384	0·002	0·000	European	UK Biobank
rs6756297	461 384	−0·003	0·000	European	UK Biobank

SNP, single-nucleotide polymorphism; MR, Mendelian randomisation.

Table 4. Description of the diabetic outcome-SNP association in the two-sample MR (Coefficient values and standard errors)

	Outcome									
	Fasting glucose					HbA1c				
SNP	n	β	SE	Population	Consortium	n	β	SE	Population	Consortium
rs4861163	133 010	0·004	0·002	European	MAGIC	46 368	−0·004	0·004	European	MAGIC
rs10822145	133 010	−0·005	0·002	European	MAGIC	46 368	0·001	0·003	European	MAGIC
rs6756297	58 074	−0·003	0·008	European	MAGIC	46 368	−0·003	0·009	European	MAGIC
	HOMA-IR					Fasting insulin				
rs4861163	37 037	0·004	0·005	European	MAGIC	51 750	0·004	0·003	European	MAGIC
rs10822145	NA	NA	NA	NA	NA	51 750	−0·006	0·004	European	MAGIC
rs6756297	37 037	0·004	0·010	European	MAGIC	51 750	−0·002	0·008	European	MAGIC

SNP, single-nucleotide polymorphism; MR, Mendelian randomisation; HOMA-IR, homeostatic model assessment for insulin resistance.
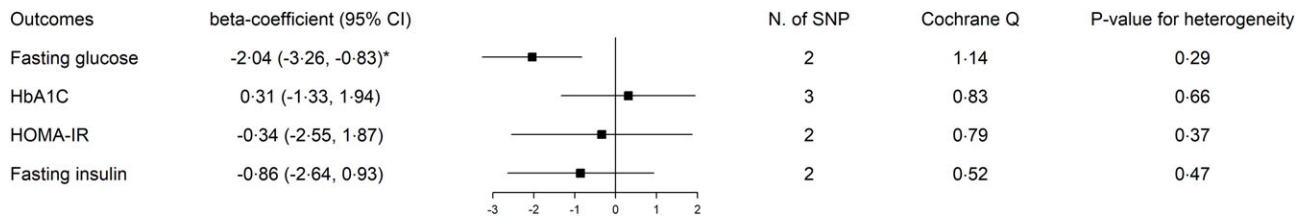
Discussion

Principal findings

This systematic review, including fourteen RCT between 2003 and 2021, reported how single Zn supplements impacted glycaemic control in T2D. In meta-analyses with random-effect models, Zn supplements were shown to lead to a significant reduction in the level of post-trial fasting blood glucose, HbA1c and HOMA-IR, but not in the serum insulin level. These results had moderate certainty of evidence. Trial duration contributed to the heterogeneity in the results of fasting blood glucose and HbA1c and diabetes type specified contributed to the heterogeneity in HOMA-IR and serum insulin level. In the two-sample MR analysis, with two SNP, Zn supplement was also significantly associated with a lower level of fasting glucose, among the general population. The difference in effect sizes between the review and two-sample MR was small (−26·52 v. −37·09, overlapping in CI).

Previous literature

The protective power of Zn supplements was demonstrated in the previous literature across different populations and diabetes types. In a cohort study encompassing 14 140 Japanese participants, the dietary intake of Zn was associated with a lower OR of T2D mellitus among the younger (age 40–55 years)[68]. The result of lower T2D risk was also consistent with an Australian women's longitudinal cohort[69]. Furthermore, de Carvalho and colleagues found a negative correlation between %HbA1c and plasma Zn levels, and women with deficient Zn levels had higher scores of HOMA-IR and C peptide values in a systematic review[70]. Besides, a study with two cohorts observed an 11 % decrease in the risk of gestational hyperglycaemia by every 1 mg/d Zn intake in women, which might be used to prevent gestational diabetes[71].

In 2019, Wang et al. indicated that Zn supplementation could significantly reduce key glycaemic indicators in a systematic

Outcomes	beta-coefficient (95% CI)		N. of SNP	Cochrane Q	P-value for heterogeneity
Fasting glucose	-2·04 (-3·26, -0·83)*		2	1·14	0·29
HbA1C	0·31 (-1·33, 1·94)		3	0·83	0·66
HOMA-IR	-0·34 (-2·55, 1·87)		2	0·79	0·37
Fasting insulin	-0·86 (-2·64, 0·93)		2	0·52	0·47

Fig. 5. Two-sample MR results with inverse variance weighting coefficient. MR, Mendelian randomisation.

review of RCT that included all diabetes types. This review involved 1700 participants and found a larger net change among fasting glucose, 2-h postprandial glucose, HbA1c and HOMA-IR in the Zn intervention arm[21]. Zn supplements had a larger effect on fasting glucose in diabetes patients than people at high risk and a preventative effect among pre-diabetes[21]. These findings were consistent with our results on Zn's favorable effect on glycaemic control and the magnitude of HbA1c's MD was similar to ours. But they did not specify the diabetes type in their population included. A systematic review of RCT published in early 2021 specified Zn as a single factor of intervention and found that low-dose and short-duration Zn supplements also showed significant improvements in some T2D indicators, compared with the high dose and long duration[22]. The duration-specific effect also supported our result in meta-regression about the heterogeneity contribution of the trial duration, which plays a critical role in the Zn supplement's effect. However, the true effect of Zn might be masked by the multi-supplement intervention or non-specified diabetes type with differential biological mechanisms.

There are not many MR studies that directly linked Zn as exposure to diabetes. An MR study in 2019 did not find any significant causal relationship between Zn level in blood and odds of T2D with two SNP[72]. The difference with our results may be due to the supplementary effect of Zn.

Strength

To our knowledge, this study is the first to use mixed methods by the systematic review of RCT and MR to triangulate evidence of causal inference in the field of nutritional supplementation. This systematic review included the newest, until 2021, literature with scientific rigor and delivered a clear statement of the Zn supplement's protection on T2D at the individual level. Our result in improving glycaemic control was consistent with previous literature. Not only that, but we also narrowed the intervention into a single supplement to emphasise the effect of Zn with less concern of contamination bias. Moreover, on the basis of the review, the application of two-sample MR minimised residual confounding or reverse causality due to imperfect randomisation or short duration and gave a more comprehensive lifelong evaluation. MR assumed that the effect of instrumental SNP was only from the exposure of interest to the outcome, independent of other factors. Compared with one-sample MR, two-sample increased statistical power with a larger population from multiple GWAS consortia[30].

Limitation of included studies, systematic review

The majority of the studies had relatively small groups in each arm (around thirty participants). Although this may limit the power and confounder adjustment, the method of meta-analysis could combine them to get stronger results. Moreover, the trials with at least some concerns of bias were not included in the main interpretation. In the sensitivity analysis which included all trials regardless of the risk of bias, the effect size was similar to the meta-analysis for low-risk only, which reduced our concern (online Supplementary Fig. 2). Suspicious publication bias of fasting blood glucose perhaps came from the small number of trials included. Given the small sample size and variation between trials, the results should be more carefully interpreted and cautiously applied in clinical practice.

Notably, some studies were excluded from the primary analyses for only reporting post-trial means or change scores, which could induce publication bias. For example, Balk et al. analysed the within-group correlation from 123 studies and gave the median correlation values of 0·54 in the treatment group and 0·73 in the control group[73]. Using the equation for change scores' SE from the Cochrane handbook[74], we calculated all the possible change scores and their SE of HbA1c and performed another sensitivity meta-analysis (online Supplementary Fig. 4). Zn intervention led to a large decrease in HbA1c than the control group. Thus, our initial conclusion appears robust after this sensitivity analysis.

Also, there may exist the problem of generalisability that most of the trials were in Asia, which may induce the problem of extrapolation. A possible explanation is that researchers from high-income countries prefer to perform trials with co-supplement and target a wider population of all types of diabetes. Moreover, inadequate Zn intake was more common in developing countries than in developed countries[75,76]. Zn deficiency was also found to aggravate insulin resistance and hyperglycaemia[77,78], therefore researchers from a developing country may prefer to choose Zn supplementary as a single intervention. Moreover, it is possible that the improvement in glycaemic control was due to reducing pre-existing Zn deficiencies within the study population, so that the benefits in the general population may disappear. However, our results were consistent with previous reviews which included studies from developed countries like Australia or USA, which may relieve the problem to some extent[21,22,79]. Thus, further RCT with larger sample sizes in a variety of geographic regions would be preferred.

Limitation of review processes, systematic review

In the review process, the study was limited in that only one reviewer screened every paper and extracted information. The high percentage agreement and Gwet AC in the random set reduced our concerns. Second, we found some degree of heterogeneity in each outcome analysis. Due to the limited number of trials, the meta-regression was not strong enough to detect explanatory variables. We may expand our criteria to include more trials in the situation of not affecting the directness. Moreover, we were aware that the risk of type I error would increase with multiple tests in our study.

Limitation of two-sample Mendelian randomisation

The MR part was limited to the low number of SNP included. This meant that sensitivity analysis for exclusion restriction assumption and leave-one-out could not be used. Since HbA1c has three SNP in the two-sample MR, the sensitivity analyses were performed, and the results were all non-significant (online Supplementary Table 3). However, MR studies are often low-powered, and the small number of SNP will have additionally reduced the power of the MR analyses, so we did not present other outcomes' results from the MR-Egger regression, weighted median and weight mode analyses[80]. Although there is a potential bias from the effect of supplementary, the negative control using Ca supplementation implied that this is unlikely to have produced a substantive bias (online Supplementary Table 4). The two-sample MR results of negative control: hair colour suggested no significant association between Zn supplementary and hair colour (online Supplementary Table 5), which reduced the threat from bias due to residual population structure. Third, pooled analysis of individual studies made us not access the individual patient data to specify the T2D population.

Biological plausibility

Zn's antioxidant properties are the biologically plausible connection to T2D. Oxidative stress reflected an imbalance between the production of reactive oxygen species and antioxidant defenses. Excess reactive oxygen species cause lipid peroxidation, leading to the lesion of cell membranes and lipoproteins, which ultimately damages insulin secretion and increases insulin resistance through the signaling pathway within the β-cells of pancreatic islets[81,82]. Plasma advance oxidation protein products generated during oxidative stress were indicated as a biomarker of endothelial dysfunction as early events for T2D diabetes[83]. As a catalytic role for Zn superoxide dismutase, Zn involves the conversion of superoxide radicals to molecular oxygen and hydrogen peroxide through antagonism, which could reduce reactive oxygen species toxicity[84,85]. For chronic effects, Zn is also an inducer of metallothionein in multiple organs[85]. The metallothionein immunoreactivity levels were higher in the tubular areas of the Zn-supplemented group, suggesting zinc's stimulation effect to regulate the oxidative stress in the rat[86]. The sulfhydryl stabilisation by Zn protection was another mechanism against oxidative damage[87].

Many studies have investigated the metabolism of Zn in the pathogenesis of diabetes mellitus. In the spontaneously diabetic mice, researchers observed the *in vitro* insulinomimetic activity, hypoglycaemic effect in glucose and insulin resistance attenuation by administration of Zn complexes[88]. In the population study, diabetic patients had a higher level of 8-hydroxy-2-deoxyguanosine, oxidative damage to DNA and a lower level of Zn, which suggested more serious oxidative lesions[89]. By analogy, people with higher dietary antioxidant capacity (richer antioxidants in diet) were associated with a lower risk of T2D and a lower score of HOMA-IR across sexes in the Rotterdam study[90]. Antioxidants become therapeutic options to manage diabetes and its serious complications such as Metformin, which reduces the production of reactive oxygen species and increases insulin sensitivity[91]. So, Zn administration has huge potential to be a first-line intervention treatment for T2D due to its antioxidant mechanism.

Implications

Optimal ways to utilise the Zn supplement intervention in real-world health practices need more concrete explorations in the particular context of disrupted routine care due to the COVID-19 pandemic. According to a rapid WHO assessment, nearly 50 % of diabetes and diabetic complications management services were partially or completely disrupted, which was an essential service in most of countries, especially among low- and middle-income countries[92]. Timely nutritional interventions are essential for successful and sustainable diabetes care to overcome the negative effect from the implication of strict infection prevention and control[93]. Self-care practice should be encouraged during this extreme health workforce shortage[94]. Immediate implementation of Zn as an efficient tool or complement is needed for patients who struggle with limited medical resources and primary care systems, which are considerably disrupted. Nevertheless, we should be discreet about the dose of Zn supplements. According to the Food and Nutrition Board, USA, the recommendations of Zn daily intake for adult males and females are 11 mg and 8 mg[95]. Moreover, Gibson et al. reviewed the dietary Zn recommendations of multiple international agencies and organisations and suggested around 40 mg/d of tolerable upper intake level of Zn for adults[96]. The Zn supplementary suggestion should be given in the consideration of recommended amount and daily diet.

Conclusion

The study found that a single Zn supplement was strongly associated with a lower level of glycaemic indicators in T2D, suggesting a protective effect. The systematic review and two-sample MR supported a causal association with some evidence but need to be further validated due to fewer studies and SNP included, so the interpretation of the results should be careful. Overall, we advocate the use of Zn supplements, within the proper guideline, as an efficient nutritional intervention to support routine diabetic care.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522002616

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