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Dietary zinc intake and whole blood zinc concentration in subjects with type 2 diabetes versus healthy subjects: A systematic review, meta-analysis and meta-regression

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Keywords: Zinc; type 2 diabetes mellitus; review; EURRECA.

Abbreviations: T2DM, type 2 diabetes mellitus; EURRECA, EURopean micronutrient RECommendations Aligned; MD, mean difference; AAS, absorption spectrophotometry; ICP-MS, inductively coupled plasma mass spectrometry; ICP-AES, inductively coupled plasma atomic emission spectrometry.

Abstract

The aim of this systematic review, meta-analysis and meta-regression was to examine the relationship between type 2 diabetes mellitus (T2DM) and concentration of zinc in whole blood, as well as dietary zinc intake. Searches were performed using Ovid MEDLINE,

Embase (Ovid) and The Cochrane Library (CENTRAL). Observational studies conducted on diabetic and healthy adults, with data on dietary zinc intake and/or concentration of zinc in whole blood, were selected. The search strategy yielded 11,150 publications and the manual search 6, of which 11 were included in the meta-analyses. Mean difference (MD) and 95% confidence interval (CI), were calculated using the generic inverse-variance method with random-effects models. Heterogeneity was assessed by the Cochran Q-statistic and quantified by the I^2 statistic. Meta-regressions and stratified analysis were used to examine whether any covariate had influence on the results. The pooled MD for the dietary zinc intake meta-analysis was -0.40 (95% CI: -1.59 to 0.79; I^2 = 61.0%). Differences between diabetic and non-diabetic subjects became significant in the presence of complications associated with diabetes (MD = -2.26; 95% CI: -3.49 to -1.02; I^2 = 11.9%). Meta-regression showed that for each year since the diagnosis of diabetes the concentration of zinc in whole blood decreased in diabetic patients regarding healthy controls [MD (concentration of zinc in blood) = 732.61 + (-77.88303) x (duration of diabetes in years)], which is not generally explained by a lower intake of zinc.

Highlights

Duration of T2DM is associated with a reduction in whole blood zinc concentration.

Dietary zinc intake seems not to explain differences in whole blood zinc concentration.

Only diabetics with complications seem to consume less zinc than healthy subjects.

Introduction

Zinc is an essential trace element that is directly implicated in the physiology of glucose metabolism, participating in the synthesis, storage, secretion, action and translocation of insulin into the cells [1–3], as well as translocation of GLUT4 to the cell surface for glucose transport into the insulin responsive cells [4]. Disturbances in zinc homeostasis may play a role in the pathogenesis [5,6], pathophysiology [7–9] and control [10–12] of type 2 diabetes mellitus (T2DM). In addition, it has been observed that T2DM patients with a good glycemic control had a higher zinc status compared to those with poor glycemic control [13]. T2DM is a major global health problem. The prevalence of diabetes worldwide was estimated as 415 million people aged 20-79 years in 2015, and it has been forecast to increase to 642 million in 2040 [14].

Several studies [15,16], but not all [17,18], have found a significantly lower concentration of zinc in whole blood in patients with T2DM compared with healthy subjects. Although blood zinc is not a sensitive biomarker of zinc status, red blood cells are rich in zinc, and changes in the concentration of zinc in whole blood may reflect alterations in red blood cell zinc transport. It has been proposed that a lower dietary zinc intake in diabetic individuals may

explain the reduced plasma and whole blood zinc concentrations observed in some studies [19,20]. This seems to be supported by a dose–response relationship between zinc intake and zinc status in adult populations observed in a systematic review and meta-analysis [21]. However, differences in dietary zinc intake between patients with T2DM and healthy controls have not been confirmed by subsequent studies [22,23].

The purpose of this study was to undertake a systematic review, meta-analysis and meta-regression of the published literature to examine the relationship between T2DM, concentration of zinc in whole blood, and dietary zinc intake.

Material and methods

This systematic review and meta-analysis was conducted within the framework of the EURopean micronutrient RECommendations Aligned (EURRECA) Network of Excellence. It has been recorded in PROSPERO (2015: CRD42015020178) and can be consulted here: (http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42015020178). The implementation of this systematic review and meta-analysis was performed following the MOOSE criteria statement [24].

Literature search

The search strategy was performed as part of a wider review process to identify studies assessing the effect of zinc on different outcomes. The searches were performed of literature published up to and including March 2016 in Ovid MEDLINE, Embase (Ovid) and The Cochrane Library (CENTRAL) using search terms for ('study designs in humans') AND (Zinc) AND (intake OR status). Manual searches of references supplemented the electronic search.

Study selection

To be included in the research, studies had to be original publications of observational design (prospective cohort, case-control, and cross-sectional), conducted on human adults (≥18 years) with type 2 diabetes mellitus (T2DM) patients and healthy individuals as controls. In addition, included studies needed to report data on the mean and standard deviation of dietary zinc intake and/or concentration of zinc in whole blood in both diabetic and non-diabetic subjects. Animal and in vitro studies, studies with diabetic participants other than T2DM, and study designs other than prospective cohort, case-control, and cross-sectional studies, were excluded.

The selection process was carried out in two stages, the first involved filtering studies by their title and abstract (Figure 1). In the second stage, potentially relevant studies were

explored further using the full text of the article and assessed against the inclusion and exclusion criteria. Stages 1 and 2 were carried out by members of the team (JCFC, MWM, VHM, CD and NL). A 10% sample was cross checked by a second researcher to ensure consistency between reviewers and any discrepancies were resolved by discussion.

From each article selected for inclusion at stage 2, the following data were extracted into an excel spreadsheet: author, publication year, study design, sample size, control matched, geographical area, gender, age, BMI (Body Mass Index), complications associated with diabetes, duration of T2DM, dietary survey method used to estimate zinc intake, number of days of administration of the dietary survey, dietary zinc intake of participants, laboratory technique used to determine concentration of zinc in whole blood, and its concentrations (Table 1). The extracted data were checked independently by at least two researchers. In order to incorporate relevant data from those selected studies that presented results in forms other than the mean and standard deviation, such as median and the interquartile range, we used the estimation methods proposed by Wan et al. [25]. These methods allowed appropriate estimates to be made for both normal data and skewed data. Variables expressed as a range, such as age in diabetic and non-diabetic groups, duration of diabetes in diabetic subjects, were assigned the midpoint of the range to estimate a mean value. The quality of publications (Table S1) was evaluated using the STROBE Statement [26], and the score was used in the statistics analyses. If any of the data was missing, the authors were contacted for additional data.

Statistical analysis

Data were expressed as mean difference (MD) with 95% confidence interval (CI), and pooled using the generic inverse-variance method with random-effects models. MD in both meta-analyses was calculated as the difference between the values of diabetics and those of non-diabetics. Forest plots were created to visualise summary estimates. Heterogeneity was assessed by the Cochran Q-statistic and quantified by the I^2 statistic, which represents the percentage of variation attributable to between-study heterogeneity [27]. I^2 values of 25%, 50%, and 75% were considered as low, medium, and high heterogeneity, respectively [28,29]. Potential sources of heterogeneity in both meta-analyses were explored by univariate meta-regressions (tables 2 and 3), using covariates such as geographic area, eastern (1) vs. western (2); continent, America (1), Asia (2), Oceania (3), and Europe (4); gender, men (1) vs. women (2); quality (STROBE Statement [26]), both categorical and continuous variables; age of diabetics patients and healthy controls, both categorical and continuous variables; BMI category in diabetic and non diabetic subjects, normal weight (18.5-24.9 kg/m^2) (1) vs. overweight (25.0-29.9 kg/m^2) (2), duration of diabetes, both

categorical and continuous variables, complications associated with diabetes, without (1) vs. with complications (2); sample size, both categorical and continuous variables; matched, yes (1) vs. no (2);dietary survey method, 24-hour dietary recall (1) vs. dietary record (2); number of days of administration of the dietary survey, 1, 2, 3, 4 and 7 days and laboratory technique zinc assessment method to determine the concentration of zinc in whole blood, atomic absorption spectrophotometry (AAS) (1), inductively coupled plasma mass spectrometry or atomic emission spectrometry (ICP-MS or ICP-AES) (2).

Multivariate meta-regressions were used to examine the covariates that had a significant influence on heterogeneity in univariate analysis. Covariates showing collinearity were removed from the final multivariate model. In addition, to examine how much of the heterogeneity was accounted for by the studied covariates, the adjusted R^2 was calculated by comparing the baseline value of the heterogeneity variance obtained from the empty regression model, with the heterogeneity variance from the meta-regression after the covariate was added. Finally, stratified analysis was also used in order to assess possible causes of heterogeneity.

Random effects meta-regressions were also used to examine whether any covariate had influence on the results. Bubble plots were created to show a relevant influence of a single continuous covariate on MDs in a meta-regression model. This graph represents the fitted regression line together with circles representing the estimates from each study, sized according to the precision of each estimate (the inverse of its within-study variance). Publication bias was investigated by visual inspection of funnel plots and quantitatively assessed using Egger's [30] and Begg's [31] tests.

To test the influence of each study on the overall effect size, sensitivity analysis was conducted using Leave-One-Out method, that is removing one study each time and repeating the analysis [32]. All analyses were conducted in STATA statistical software (Version 12.0. STATA Corp., College Station, Texas, USA).

Results

The search strategy initially yielded 11,156 publications (Figure 1), of which 11 were included in the meta-analyses [15–20,22,23,33–35]. Nine results were included for the final meta-analysis that compared the dietary zinc intake in diabetic and non-diabetic subjects [19,20,22,23,33,34]. Meanwhile, six results were included in the meta-analysis that compared the concentration of zinc in whole blood in diabetic and non-diabetic subjects [15–18,35]. The characteristics of the included studies are summarized in Table 1.

Meta-analysis of dietary zinc intake and T2DM data: Four studies selected for the dietary zinc intake meta-analysis were carried out in western countries (England [33], Brazil [20,22] and Australia [23]) and two in eastern countries (Korea [34] and Iraq [19]). All those studies had cross-sectional design [20,22,23,33,34], except a clinical trial performed by Al-Maroof and Al-Sharbatti in Iraq [19]. However, data extracted for our systematic review and meta-analysis were collected at one time point, before intervention, so we were able to include baseline data from the study of Al-Maroof and Al-Sharbatti. Dietary zinc intake was determined using two different survey methods, 24-hour dietary recall, used by both studies from western countries [19,34], and dietary record administered for two [23], three [22], four [20] or seven [33] days. Two studies recruited only women [23,34], while the other studies collected data in men and women [19,20,22,33]. Known duration of diabetes was 7.20±7.26 years for diabetic participants, taking into account five of the six selected studies [19,20,22,23,34].

Meta-analysis of the concentration of zinc in whole blood and T2DM: Two studies selected for this meta-analysis (Table 2) were conducted in western countries (Austria [17] and Italy [18]), and three in eastern countries (Pakistan [15,35] and China [16]). Their design was cross-sectional, and the laboratory method used for tissue zinc analysis was AAS [15,17,35] or ICP-MS or ICP-AES [16,18]. The time since diagnosis of T2DM in patients ranged from 8 to 20 years. The quality of selected studies was moderate, according to the STROBE Statement [26].

An overall pooled MD of -0.40 (95% CI: -1.59 to 0.79) was observed in the meta-analysis that investigated the dietary zinc intake in diabetic cases and healthy controls (Figure S1). Differences become significant (MD = -2.26; 95% CI: -3.49 to -1.02) when diabetes was associated with complications (Figure 2). Moderate heterogeneity was observed in this meta-analysis (I^2 = 61.0%, P = 0.009), suggesting that 61% of the variability between studies was due to differences in dietary zinc intake and 29% due to sampling variation. To examine how much of the heterogeneity was accounted for by the presence of complications associated with diabetes, we used the adjusted R^2 (Tabla 2), and observed that this explained heterogeneity largely (R^2 = 92.20%; I^2 residual = 0.00%). Thus, when we stratified by the presence of complications associated with diabetes, we observed that heterogeneity was reduced to 0.0% in the group without complications, and to 11.9% in the group with complications (Figure 2). In addition to complications associated with diabetes, duration of diabetes contributed significantly to the heterogeneity (R^2 = 100.00%; I^2 residual = 0.00%). Through meta-regression (Figure 3), we found that for each year since the diagnosis of diabetes the mean difference in dietary zinc intake between diabetics and non-diabetics

subjects seemed to increase [MD (dietary zinc intake) = 1.67 + (-0.37) x (duration of diabetes in years)].

In the meta-analysis that compared the concentrations of zinc in whole blood in diabetic and non-diabetic subjects, a pooled MD of -216.80 (95% CI: -359.42 to -74.19) was found (Figure S2). High heterogeneity was found in this meta-analysis (I^2 = 97.7%, P < 0.001). However, stratified analyses by geographic area, western vs. eastern countries, reduced considerably the heterogeneity (I^2 = 47.1%, P < 0.129) and (I^2 = 69.3%, P < 0.071), respectively (Figure 4). Identical results were obtained when we stratified by studies which had been matched by age vs. not matched by age (Figure S3), had similar aged groups in cases and controls vs. those which had different ages groups (Figure S4), and with sample size higher vs. lower than 150 participants (Figure S5). Furthermore, these stratified analysis explained 96.24% of heterogeneity R^2 = 96.24%; I^2 residual = 55.19%). In this meta-analysis, duration of diabetes had also an important influence on heterogeneity (R^2 = 96.16%; I^2 residual = 58.23%). We have also observed that for each year since the diagnosis of diabetes, the concentration of zinc in whole blood (1µg/dL = 0.0483µmol/L) is reduced in diabetic patients regarding healthy controls. This data is presented as a bubble plot (Figure 5), and expressed in the equation of the regression line [MD (concentration of zinc in blood) = 732.61 + (-77.88303) x (duration of diabetes in years)]. Finally, sample size also had an impact on the results (Figure 6) [MD (concentration of zinc in whole blood) = 371.84 + (-3.44) x (sample size)].

By visual inspection, an overall symmetry of the funnel plots was observed for all meta-analyses (Figures S6 and S7). This was confirmed by the Egger's (P=0.553 for meta-analysis of dietary zinc intake, and P= 0.768 for meta-analysis of the concentration of zinc in whole blood) and Begg's (P=1.000 for both meta-analyses) tests which were not significant, indicating the absence of publication bias. In addition, neither of the two meta-analyses results were affected substantially after removing one study at a time and repeating the analysis. The combined result ranged between -0.70 (95% CI: -1.87 to 0.46), after removing the result of Rauscher et al. [33] in men, and -0.08 (95% CI: -1.35 to 1.19), after removing the study of Lee et al. [34] for the meta-analysis of dietary zinc intake. Meanwhile, for the meta-analysis of the concentration of zinc in whole blood, results ranged between -187.76 (95% CI: -344.87 to -30.65), after removing the result of Kazi in women, and -264.86 (95% CI: -389.79 to -139.93), after removing the study of Ekmekcioglu [17].

Discussion and Conclusion

Data from this systematic review, meta-analysis and meta-regression of observational studies suggests that there are significant differences in the concentration of zinc in whole

blood between diabetic patients and healthy subjects. In addition, the duration of diabetes appears to be associated with the concentration of zinc in whole blood, which is not explained by a lower of dietary zinc intake in diabetic patients. Only when T2DM is associated with complications, such as diabetic nephropathy in which dietary therapy is essential, differences in dietary zinc intake became significant. Changes in the concentration of zinc in whole blood may reflect changes in tissue zinc distribution and cellular zinc homeostasis in patients with diabetes, as was suggested by Chausmer in 1998 [36].

Our results did not show significant differences in dietary zinc intake between diabetic and non-diabetic subjects. However, the combined result showed some evidence of heterogeneity (I^2 = 61.0%). When we stratified by the presence of complications associated with diabetes, we found significant differences in the subgroup with complications (MD = -2.26; 95% CI: -3.49 to -1.02), and low heterogeneity (I^2 = 11.9%). Meanwhile, in the subgroup without complications no significant differences in dietary zinc intake between groups, and an undetectable heterogeneity (I^2 = 0.0%) were found (Table 2). Only two studies reported complications associated to T2DM in diabetic participants [20,34]. The study of Batista et al. was conducted on diabetic subjects with chronic kidney disease due mainly to diabetic nephropathy [20]. Meanwhile, Lee et al. reported that only 24.0% of T2DM cases did not have associated complications, 36.0% presented diabetic nephropathy, and 10% coronary heart disease [34]. In patients with diabetic nephropathy, diet therapies, such as protein restriction are commonly advised [37,38]. It is well known that zinc intake correlates directly with protein intake, as these two nutrients share the same food sources [39]. This correlation was also observed in the study carried out by Batista et al. [20], supporting the role of advanced kidney disease as a cause of a lower dietary zinc intake in this patients. The change from not having to having complications associated with diabetes supposes a significant increment in MD of dietary zinc intake of 2.70 mg/day (MD = -2.70; 95% CI: -4.48 to -0.93; P = 0.009). Thus, diabetic patients consume 2.70 mg/day less zinc than healthy controls when they have associated pathologies (Table 2).

The duration of diabetes seems to have an influence on dietary zinc intake. We observed that stratified analysis by duration of diabetes (<8; ≥8 years) and (<5; 5-8; ≥8 years) reduced the heterogeneity considerably. In addition, when we conducted a meta-regression, introducing duration of diabetes as a continuous variable, we found that for each year since the diagnosis of T2DM, diabetics consume less zinc [MD (dietary zinc intake) = 1.67 + (-0.37) x (duration of diabetes in years)] in their diet (Figure 3). However, this may be due to the inclusion of the studies where the diabetic patients had complications as described above. When these studies are excluded, no significant relationship or trend between the

duration of diabetes and the MD of dietary zinc intake was observed (MD = 0.07; 95% CI: -2.79 to 2.94, P = 0.924).

Variables, such as gender, geographic area, age or BMI, do not appear to have an impact on the results (Table 2), suggesting that dietary zinc intake is similar in diabetic and non-diabetic subjects in all age ranges, in both genders, in normal weight or overweight, and in different populations.

For all the above, the dietary zinc intake does not seem to explain the observed differences in the concentration of zinc in whole blood between diabetic and non-diabetic individuals. However, these differences (Figure S2) may not be reliable due to the very high heterogeneity observed (I^2 = 97.7%). Stratified analysis by geographic area (Figure 4), as well as, matched controls (Figure S3), older group (Figure S4) or sample size (Figure S5), significantly reduced the heterogeneity to 47.1% and 69.3% in each subgroup (Table 3), demonstrating their influence on the differences in the concentration of zinc in whole blood, and making the result more plausible.

We have also observed significant differences in the concentration of zinc in whole blood between cases and controls, but only in eastern countries (Figure 4), namely China [16] and Pakistan [15,35] (table 3). Studies conducted in Austria [17] and Italy [18], did not show significant differences in the concentration of zinc in whole blood between cases and controls. Studies from eastern countries compared diabetic patients with age-matched healthy controls, whereas, studies from western countries recruited patients and controls with significantly different ages, which may explain differences in results found according to the geographic area.

The influence of the duration of diabetes on the concentration of zinc in whole blood has also been examined (Figure 5). The results revealed that for each year since the onset of T2DM, the concentration of zinc in whole blood decreases compared to healthy subjects. This result is consistent with study conducted by Luo et al., who found that duration of diabetes was a key determinant of serum zinc levels [40], suggesting that the duration of diabetes may be associated with changes in zinc homeostasis. Drug therapy in diabetic patients could play a key role in the relationship between the duration of diabetes and the concentration of zinc in whole blood. Thus, it has been proposed that insulin treatment may normalised the tissue zinc concentration [9]. Unfortunately, this premise could not be assessed in our meta-analysis since data from selected studies was insufficient. Only two studies reported the use of insulin therapy in 40% [15] and 100% [35] of diabetic participants, and their whole blood zinc concentrations were significantly lower compared to

healthy controls. These data do not support the hypothesis, but further studies are needed to explore this in more detail.

Some limitations to our study need to be considered. First, the relatively small number of studies included in both meta-analyses, despite the large number of articles obtained from the search (11,156). This is because the robust search strategy within the framework of the EURRECA Network of Excellence was conducted as part of a wider review process to identify studies assessing the effect of zinc on different outcomes, and was designed to avoid publication bias. In addition, we included non-English language journals, reducing any potential bias. Furthermore, standard tests and visual inspection of funnel plots in both meta-analyses suggest that there is little evidence for publication bias. Second, the quality of the selected studies was moderate, according to the STROBE Statement [26]. After evaluating the influence of quality on results through stratified analyses and meta-regression, no significant impact on the effect size was observed in any of the meta-analyses. Furthermore, when the highest quality studies were combined to obtain a pooled MD, there was no detectable heterogeneity in either meta-analysis (0.0%), and the results were similar to the respective overall pooled MD (Tables 2 and 3). Third, since the overall analyses did not have a large sample size, the stratified analyses might have insufficient power to detect potential sources of heterogeneity. In order to correct this, we carried out, not only stratified analysis, but also random effects meta-regressions to examine whether any covariate had influence on the results. Fourth, the results showed an undetectable to moderate heterogeneity in both meta-analyses (Figures 2 and 4), which also contribute to the study validity. Finally, none of the selected studies for meta-analysis of zinc concentration reported whether or not the samples were collected from fasting participants. This could be a source of bias, however it is noteworthy that all the selected studies for this meta-analysis used whole blood samples to measure zinc concentration, thus avoiding possible biases caused by using different samples.

The relationship between zinc and glucose metabolism has been evidenced in numerous studies. Zinc participates in the synthesis, storage, secretion, action of insulin and translocation of insulin into the cells [1–3], as well as translocation of GLUT4 to the cell surface for glucose transport into the insulin responsive cells [4]. However, the underlying biological mechanism whereby T2DM could have an impact on zinc homeostasis has not been elucidated. Recently, the importance of the cellular zinc transport system in T2DM has been highlighted [41,42]. A study conducted by Chu et al., found differences in gene expressions for most zinc transporters analysed, such as ZnT1, ZnT5, ZnT7, ZIP1, ZIP3, ZIP7, ZIP10, metallothionein-1A and metallothionein-2A. A lower gene expression in all these transporters was observed in diabetics compared to controls, suggesting disturbances

T2DM-associated zinc dyshomeostasis at the cellular level [11]. This alteration on zinc metabolism may explain differences in the concentration of zinc in whole blood in diabetic subjects observed in the present systematic review, meta-analysis and meta-regression. Nevertheless, the preliminary results of another systematic review and meta-analysis of more than 73 papers conducted by our group reveals a lack of significant difference in zinc status, measured using serum or plasma zinc concentration, between diabetic patients and healthy controls. A possible explanation to both results could be that T2DM may not induce a lower zinc status compared with non-diabetic subjects, but has a negative impact on zinc homeostasis that would lead to differences in tissue zinc concentrations, such as blood.

In conclusion, data from this systematic review, meta-analysis and meta-regression of observational studies suggests that there are significantly lower concentration of zinc in whole blood of diabetic patients compared with non-diabetic subjects, which is not explained by lower dietary zinc intakes. Only when T2DM is associated with complications, such as diabetic nephropathy, in which dietary therapy is essential, a lower dietary zinc intake is observed. However, the duration of diabetes could have a significant impact on whole blood zinc concentration. Additional research is required to elucidate the possible underpinning mechanisms.

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Declarations of interest

None

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Figure 1. Flowchart of the search for studies and the selection process.

Figure 2. Forest plot of pooled mean difference of dietary zinc intake according to the presence of complications associated to diabetes. Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure 3. Bubble plot with a fitted meta-regression line of the mean difference in dietary zinc intake and duration of diabetes. Circles are sized according to the precision of each estimate (the inverse of its within-study variance) with larger bubbles for more precise estimates.

Figure 4. Forest plot of pooled mean difference of concentration of zinc in whole blood according to geographic area. Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure 5.Bubble plot with a fitted meta-regression line of the mean difference in concentration of zinc in whole blood and duration of diabetes. Circles are sized according to the precision of each estimate (the inverse of its within-study variance) with larger bubbles for more precise estimates.

Figure 6. Bubble plot with a fitted meta-regression line of the mean difference in concentration of zinc in whole blood and sample size of the studies. Circles are sized according to the precision of each estimate (the inverse of its within-study variance) with larger bubbles for more precise estimates.

Appendix A. Supplementary data

Table S1. Evaluation of the quality based on the STROBE Statement.

Figure S1. Forest plot of pooled mean difference of dietary zinc intake. Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure S2. Forest plot of pooled mean difference of concentration of zinc in whole blood. Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure S3. Forest plot of pooled mean difference of concentration of zinc in whole blood according to control matched (No matched, matched by age). Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure S4. Forest plot of pooled mean difference of concentration of zinc in whole blood according to age in diabetic and non-diabetic groups (same age, different age). Squares represent the mean difference (MD) for each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure S5. Forest plot of pooled mean difference of concentration of zinc in whole blood according to sample size (<150, >150). Squares represent the mean difference (MD) for

each study and the size of the square reflects the study-specific statistical weight. Horizontal lines indicate the 95% CI of each study. Diamond represents the combined MD estimate with corresponding 95 % CI. I-squared and p-value inform about heterogeneity among studies.

Figure S6. Funnel plots of publication biases of studies included in the meta-analysis of dietary zinc intake. Y-axis stands for the standard error of the mean difference of dietary zinc intake and X-axis stands for the mean difference of dietary zinc intake. Each dot stands for an individual study. Asymmetric funnel plot indicates a publication bias.

Figure S7. Funnel plots of publication biases of studies included in the meta-analysis of concentration of zinc in whole blood. Y-axis stands for the standard error of the mean difference of concentration of zinc in whole blood and X-axis stands for the mean difference of concentration of zinc in whole blood. Each dot stands for an individual study. Asymmetric funnel plot indicates a publication bias.

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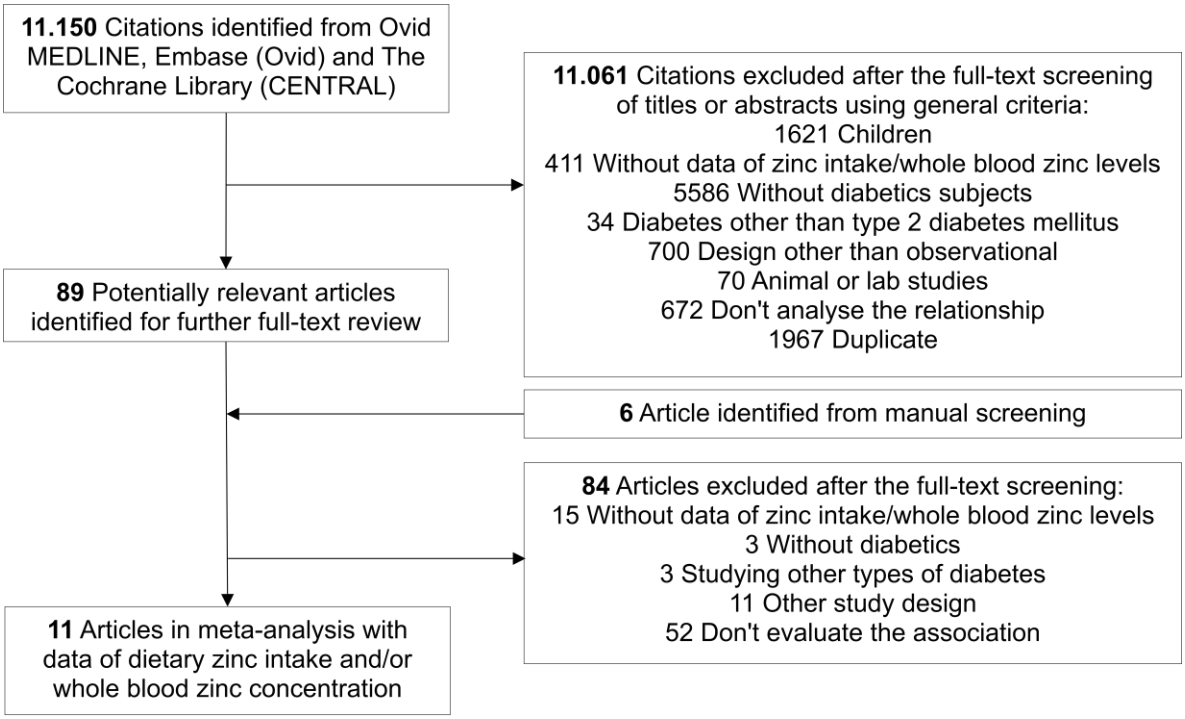

11.150 Citations identified from Ovid MEDLINE, Embase (Ovid) and The Cochrane Library (CENTRAL)
11.061 Citations excluded after the full-text screening of titles or abstracts using general criteria:
1621 Children
411 Without data of zinc intake/whole blood zinc levels
5586 Without diabetics subjects
34 Diabetes other than type 2 diabetes mellitus
700 Design other than observational
70 Animal or lab studies
672 Don't analyse the relationship
1967 Duplicate
89 Potentially relevant articles identified for further full-text review
6 Article identified from manual screening
84 Articles excluded after the full-text screening:
15 Without data of zinc intake/whole blood zinc levels
3 Without diabetics
3 Studying other types of diabetes
11 Other study design
52 Don't evaluate the association
11 Articles in meta-analysis with data of dietary zinc intake and/or whole blood zinc concentration

Table 1. Characteristics of studies included in the meta-analyses and meta-regressions on dietary zinc intake and whole blood zinc concentration in diabetic and non-diabetic subjects.

Author, year	Location	Gender	Age (years) in diabetic subjects (Mean±SD)	Age (years) in non-diabetic subjects (Mean±SD)	Sample size	T2DM (n)	Control Matched	T2DM duration in years (Mean±SD)	Zinc assessment method	Dietary (mg/day) or whole blood (µmol/L) zinc in diabetics (Mean±SD)	Dietary (mg/day) or whole blood (µmol/L) zinc in non-diabetics (Mean±SD)
Studies of dietary zinc intake											
		Women / Men	Range: 49-72 63.0	Range: 50-77 61.5	20	10				11.8±2.2	10.3±2.6
Rauscher, 1997	England	Men	Range: 49-71 61.0	Range: 50-71 58.0	10	5	Age, sex & weight	Not Showed	7-day dietary record	13.2±1.5	11.1±2.5
		Women	Range: 60-72 65.0	Range: 60-77 65.0	10	5				10.3±1.8	9.5±2.5
Lee, 2005	Korea	Women	57.9±6.9	56.3±7.9	119	50	No	8.0±6.5	24h-hour recall	6.2±2.7	8.0±3.2
Al-Maroof, 2006	Iraq	Women / Men	54.6±9.2	39.0±9.3	234	101	No	5.1±6.0	24h-hour recall	12.2±4.6	12.4±4.4
Batista, 2006	Brazil	Women / Men	61.1±8.1	52.9±9.7	53	33	No	16.3±8.8	4-day dietary record	6.5±4.2	9.4±5.0
		Men			24	16				7.5±3.8	11.6±4.5
de Sousa Lima, 2011	Brazil	Women	Range: 25-59 46.0±7.4	37.2±8.6	29	17	No	4.0±2.4	3-day dietary record	4.6±2.7	8.1±5.2
		Women / Men			73	36				12.9±5.8	11.7±4.7
		Men			22	12				16.9±7.3	14.9±6.5
Foster, 2012	Australia	Women	Range: 48.3-79 63.8±8.8	Range: 21.4-65.6 39.4±13.8	51	24				10.9±3.5	10.5±3.4
					40	20	No	6.5±5.2	2-day dietary record	12.8±5.3	11.6±3.8
Studies of concentrations of zinc in blood											
Ekmekcioglu, 2001	Austria	Not Showed	63.8±11.0	70.5±12.4	103	53	No	Not Showed	EAAS	25.7±4.7	24.9±5.8
Kazi, 2008	Pakistan	Women / Men	Range: 45-75	Range: 45-75	423	257	Age	Range: 8-20	AAS	31.3±10.6	47.9±7.5
		Men			217	137				29.9±10.7	46.9±7.2
		Women			206	120				32.7±10.4	48.9±7.7
Afridi, 2009	Pakistan	Women	Range: 30-40	Range: 30-40	182	89	Age	Range: 10-15	F/EAAS	46.9±6.3	60.4±7.2
Chen, 2012	China	Not Showed	Range: 25- >50	Range: 25- >50	158	53	Age	Not Showed	ICP- AES	43.1±31.4	60.4±48.3
Forte, 2013	Italy	Women / Men	Mean±SD: 68.4±11.2	Mean±SD: 57.2±18.0	127	68	No	Mean±SD: 10.2±8.6	ICP-MS	30.5±4.8	32.6±8.2

T2DM (Type 2 Diabetes Mellitus), SD (Standard deviation), AAS (Atomic Absorption Spectrophotometry), F/EAAS (Flame/Electrothermal Atomic Absorption Spectroscopy), EAAS (Electrothermal Atomic Absorption Spectroscopy), ICP- AES (Inductively Coupled Plasma Atomic Emission Spectrometry), ICP- MS (Inductively Coupled Plasma Mass Spectrometry).

Table 2. Stratified analysis and meta-regression for mean difference of dietary zinc intake in diabetic and non-diabetic subjects.

Subgroup	Studies (n)	Mean difference (95% CI)	Heterogeneity		Meta-regression				
			I^2 (%)	P-value	Regression coefficients (95% CI)	P-value	Tau^2	I^2 residual (%)	Adjusted R^2 (%)
Geographic area									
Eastern	2	-1.01 (-2.54; 0.53)	73.8%	0.051	-0.91 (-4.43; 2.60)	0.559	2.25	59.95%	-19.37%
Western	7	-0.10 (-1.79; 1.59)	56.1%	0.034					
Continent									
America (1)	4	-1.48 (-4.24; 1.27)	63.7%	0.041					
Asia (2)	2	-1.01 (-2.54; 0.53)	73.8%	0.051	1.01 (-0.18; 2.20)	0.085	1.06	52.24%	43.60%
Oceania (3)	1	1.20 (-1.66; 4.06)	-	-					
Europe (4)	2	1.49 (-0.37; 3.34)	0.0%	0.493					
Quality 65									
<65	5	-1.53 (-3.21; 0.15)	55.4%	0.062	2.21 (-0.28; 4.70)	0.074	0.91	41.99%	51.73%
≥65	4	0.41 (-0.56; 1.38)	3.1%	0.377					
Quality 70									
<70	7	-0.65 (-2.27; 0.96)	66.0%	0.007	0.99 (-2.80; 4.77)	0.558	2.43	62.04%	-28.84%
≥70	2	0.00 (-1.08; 1.08)	0.0%	0.374					
Gender									
Men (1)	3	-0.09 (-4.44; 4.27)	74.6%	0.019	-0.52 (-5.19; 4.16)	0.796	3.55	67.24%	-25.67%
Women (2)	5	-0.60 (-2.16; 0.95)	61.7%	0.034					
Both genders	1	-0.20 (-1.37; 0.97)	-	-	-	-	-	-	-
Age of diabetics (years)									
40-50	2	0.56 (-1.25; 2.36)	0.0%	0.606					
50-60	2	-1.01 (-2.54; 0.53)	73.8%	0.051	-0.39 (-2.64; 1.87)	0.698	2.60	65.87%	-37.75%
60-70	5	-0.51 (-2.89; 1.88)	69.3%	0.011					
Age of diabetics 50 (years)									
<50	2	0.56 (-1.25; 2.36)	0.0%	0.606	-1.41 (-5.70; 2.89)	0.465	2.27	62.33%	-20.55%
≥50	7	-0.64 (-2.02; 0.74)	67.2%	0.005					
Age of diabetics 60 (years)									
<60	4	-0.55 (-1.80; 0.70)	55.2%	0.082	-0.05 (-3.51; 3.41)	0.974	2.64	64.51%	-39.68%
≥60	5	-0.51 (-2.89; 1.88)	69.3%	0.011					
Age of non-diabetics (years)									
30-40	4	0.15 (-0.78; 1.07)	0.0%	0.723					
50-60	4	-1.62 (-3.98; 0.75)	73.5%	0.010	-0.52 (-1.96; 0.92)	0.418	2.17	58.60%	-15.08%
60-70	1	0.80 (-1.90; 3.50)	-	-					
Age of non-diabetics 50 (years)									

<50	4	0.15 (-0.78; 1.07)	0.0%	0.723	-1.57 (-4.65; 1.51)	0.267	1.86	54.22%	1.61%
≥50	5	-1.13 (-3.16; 0.89)	71.4%	0.007					
Age difference between diabetics and non-diabetics (years)									
<5	3	0.16 (-2.43; 2.76)	78.7%	0.009	0.02 (-2.15; 2.20)	0.982	2.70	62.83%	-43.24%
5-15	4	-1.48 (-4.24; 1.27)	63.7%	0.041					
≥15	2	0.00 (-1.08; 1.08)	0.0%	0.374					
Age difference between diabetics and non-diabetics II (years)									
<10	7	-0.65 (-2.27; 0.96)	66.0%	0.007	0.99 (-2.80; 4.77)	0.558	2.43	62.04%	-28.84%
≥10	2	0.00 (-1.08; 1.08)	0.0%	0.374					
Older group									
Same age (1)	3	0.16 (-2.43; 2.76)	78.7%	0.009	-0.76 (-4.26; 2.74)	0.623	2.71	64.32%	-43.85%
Different age (2)	6	-0.62 (-2.12; 0.88)	51.1%	0.069					
BMI category in diabetics									
Normal weight (18.5-24.9 kg/m^2)	5	-1.13 (-3.16; 0.89)	71.4%	0.007	1.57 (-1.51; 4.65)	0.267	1.86	54.22%	1.61%
Overweight (25.0-29.9 kg/m^2)	4	0.15 (-0.78; 1.07)	0.0%	0.723					
BMI category in non-diabetics									
Normal weight (18.5-24.9 kg/m^2)	5	-0.03 (-1.57; 1.51)	55.1%	0.064	-1.11 (-4.47; 2.24)	0.459	2.73	65.44%	-44.77%
Overweight (25.0-29.9 kg/m^2)	4	-1.10 (-3.51; 1.30)	73.6%	0.010					
Duration of diabetes (years)									
<8	4	0.15 (-0.78; 1.07)	0.0%	0.723	-2.24 (-4.00; -0.48)	0.022	0.00	0.00%	100.00%
≥8	3	-2.26 (-3.49; -1.02)	11.9%	0.321					
No data	2	1.49 (-0.37; 3.34)	0.0%	0.493	-	-	-	-	-
Complications									
Without complications of diabetes	6	0.41 (-0.41; 1.24)	0.0%	0.640	-2.70 (-4.48; -0.93)	0.009	0.15	0.00%	92.20%
With complications of diabetes	3	-2.26 (-3.49; -1.02)	11.9%	0.321					
Sample size									
<100	7	-0.10 (-1.79; 1.59)	56.1%	0.034	-0.91 (-4.43; 2.60)	0.559	2.25	59.95%	-19.37%
≥100	2	-1.01 (-2.54; 0.53)	73.8%	0.051					
Matched									
No matched	2	-0.89 (-2.14; 0.36)	58.1%	0.026	2.36 (-1.10; 5.81)	0.151	1.10	52.69%	42.20%
Matched by age, gender & weight	7	1.49 (-0.37; 3.34)	0.0%	0.493					
Dietary survey method									
24-hour dietary recall	2	-1.01 (-2.54; 0.53)	73.8%	0.051	0.91 (-2.60; 4.43)	0.559	2.25	59.95%	-19.37%
Dietary record	7	-0.10 (-1.79; 1.59)	56.1%	0.034					
Days of dietary survey									

24-hour dietary recall (1)	2	-1.01 (-2.54; 0.53)	73.8%	0.051					
2-day dietary record (2)	1	1.20 (-1.66; 4.06)	-	-					
3-day dietary record (3)	2	0.56 (-1.25; 2.36)	0.0%	0.606	0.26 (-0.45; 0.97)	0.413	1.82	58.44%	3.60%
4-day dietary record (4)	2	-3.76 (-6.17; -1.36)	0.0%	0.808					
7-day dietary record (7)	2	1.49 (-0.37; 3.34)	0.0%	0.493					

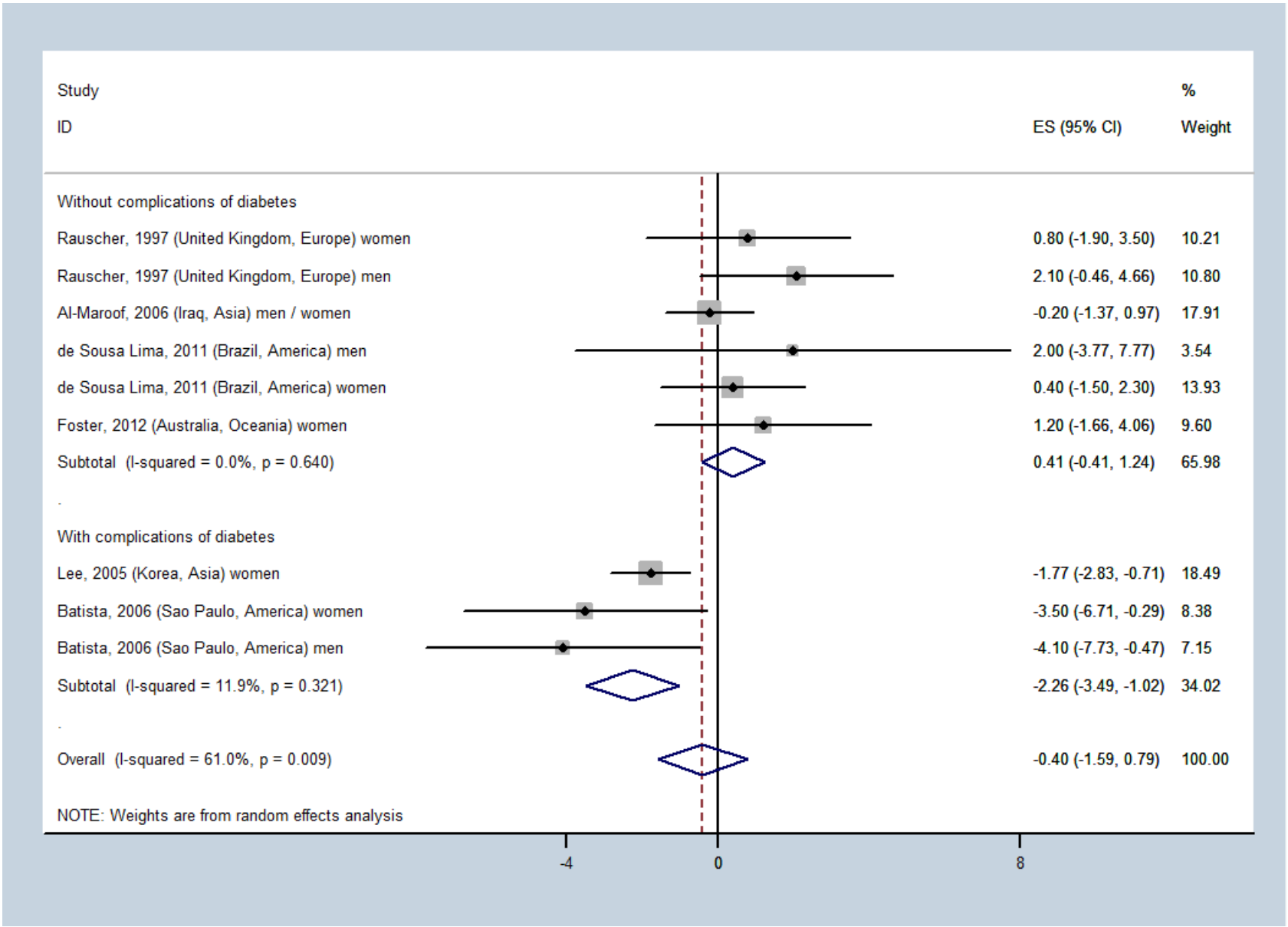
BMI (Body Mass Index).

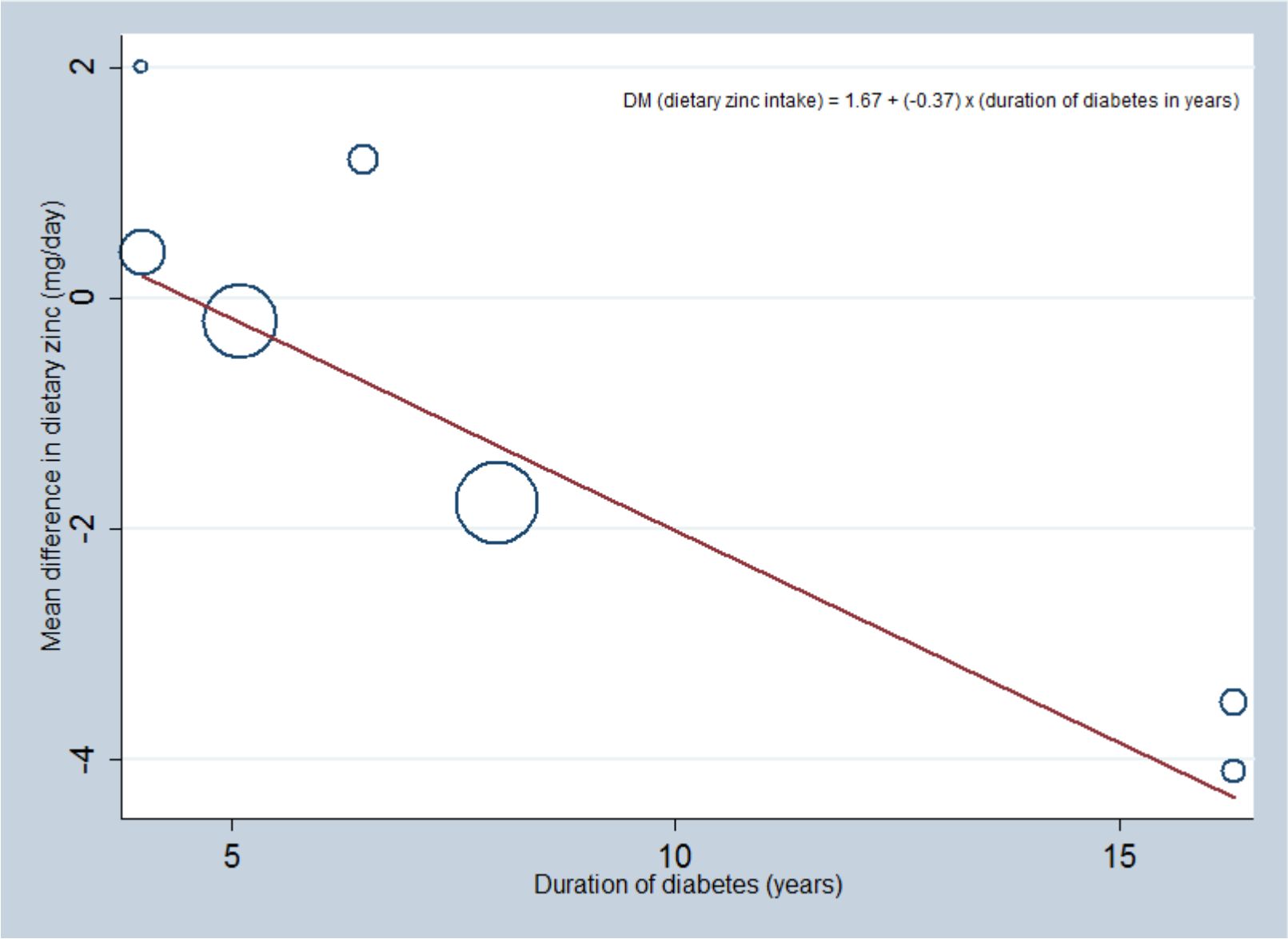
Table 3. Stratified analysis for mean difference of the whole blood zinc concentration in diabetic and non-diabetic subjects.

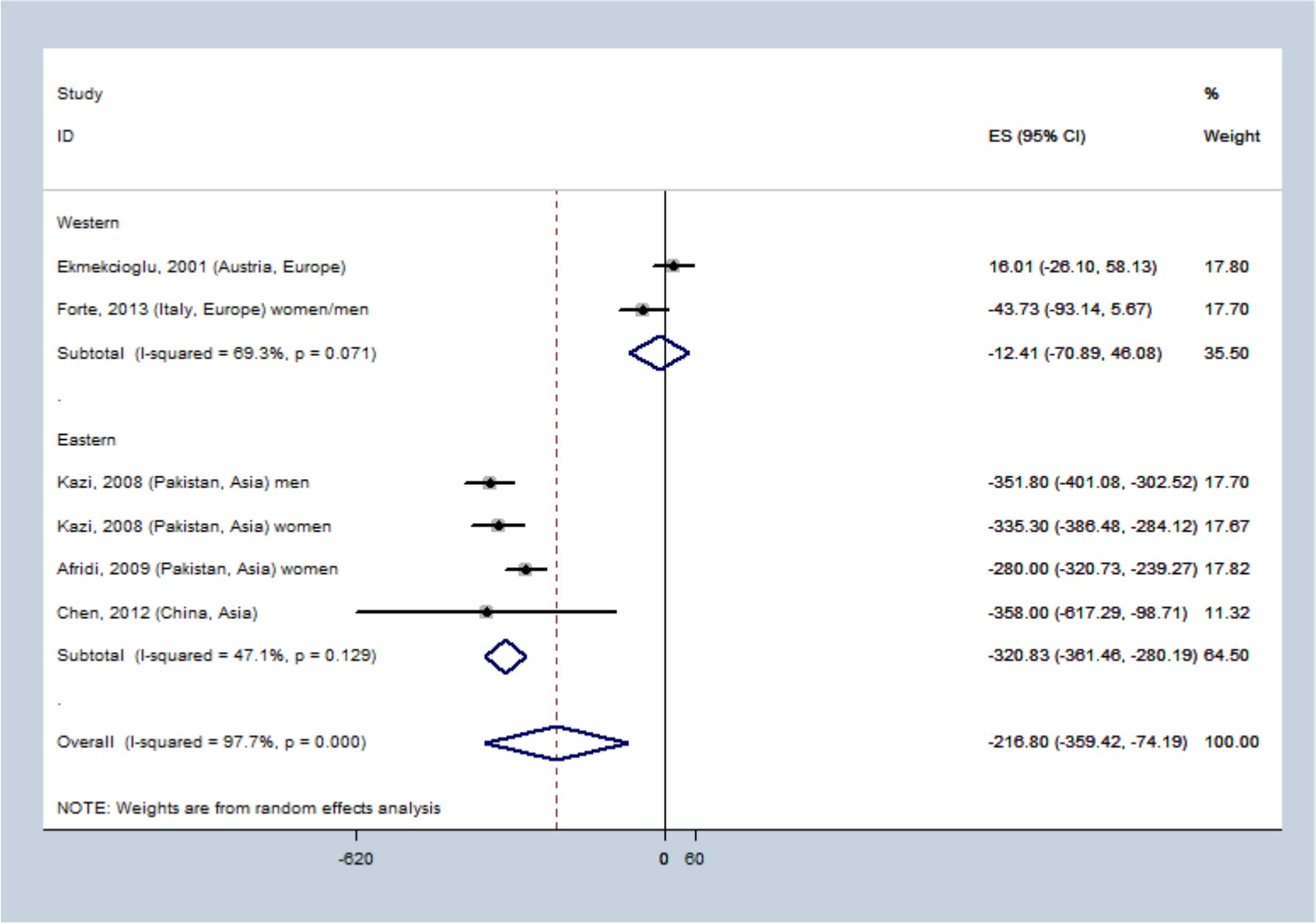
Subgroup	Studies (n)	Mean difference (95% CI)	Heterogeneity		Meta-regression				
			I^2 (%)	P-value	Regression coefficients (95% CI)	P-value	Tau^2	I^2 residual (%)	Adjusted R^2 (%)
Geographic area									
Eastern	4	-320.83 (-361.46; -280.19)	47.1%	0.129	-309.20 (-408.99; -209.42)	0.001	1012	55.19%	96.24%
Western	2	-12.41 (-70.89; 46.08)	69.3%	0.071					
Continent									
Europe	2	-12.41 (-70.89; 46.08)	69.3%	0.071	-309.20 (-408.99; -209.42)	0.001	1012	55.19%	96.24%
Asia	4	-320.83 (-361.46; -280.19)	47.1%	0.129					
Quality 50									
<50	3	-191.61 (-438.19; 54.97)	98.0%	<0.001	-55.64 (-479.08; 367.81)	0.734	32234	97.94%	-19.72%
≥50	3	-243.57 (-440.95; -46.19)	97.9%	<0.001					
Quality 55									
<55	4	-146.24 (-316.36; 23.89)	97.3%	<0.001	-202.89 (-547.55; 141.77)	0.178	18048	96.42%	32.96%
≥55	2	-343.86 (-379.36; -308.37)	0.0%	0.649					
Gender									
Men (1)	1	-351.80 (-401.08; -302.52)	-	-	46.41 (-571.35; 644.17)	0.515	972	63.59%	0.18%
Women (2)	2	-305.39 (-359.40; -251.38)	63.6%	0.097					
Both genders	1	-43.73 (-93.14; 5.67)	-	-	-	-	-	-	-
No data	2	-148.21 (-512.01; 215.58)	87.2%	0.5%	-	-	-	-	-
Age of diabetics (years)									
30-40	1	-280.00 (-320.73; -239.27)	-	-					
50-60	2	-343.86 (-379.36; -308.37)	0.0%	0.649	88.59 (-111.75; 288.93)	0.254	23307	97.58%	20.04%
60-70	2	-12.41 (-70.89; 46.08)	69.3%	0.071					
No data	1	-358.00 (-617.29; -98.71)	-	-	-	-	-	-	-
Age of diabetics 50 (years)									
<50	1	-280.00 (-320.73; -239.27)	-	-	101.57 (-580.72; 783.86)	0.668	36305	98.43%	-24.55%
≥50	4	-178.44 (-369.42; 12.55)	98.4%	<0.001					
No data	1	-358.00 (-617.29; -98.71)	-	-	-	-	-	-	-
Age of non-diabetics (years)									
30-40	1	-280.00 (-320.73; -239.27)	-	-					
50-60	3	-243.57 (-440.95; -46.19)	97.9%	<0.001	73.98 (-103.31; 251.27)	0.276	24367	97.52%	16.41%
70-80	1	16.01 (-26.58; 58.13)	-	-					
No data	1	-358.00 (-617.29; -98.71)	-	-	-	-	-	-	-

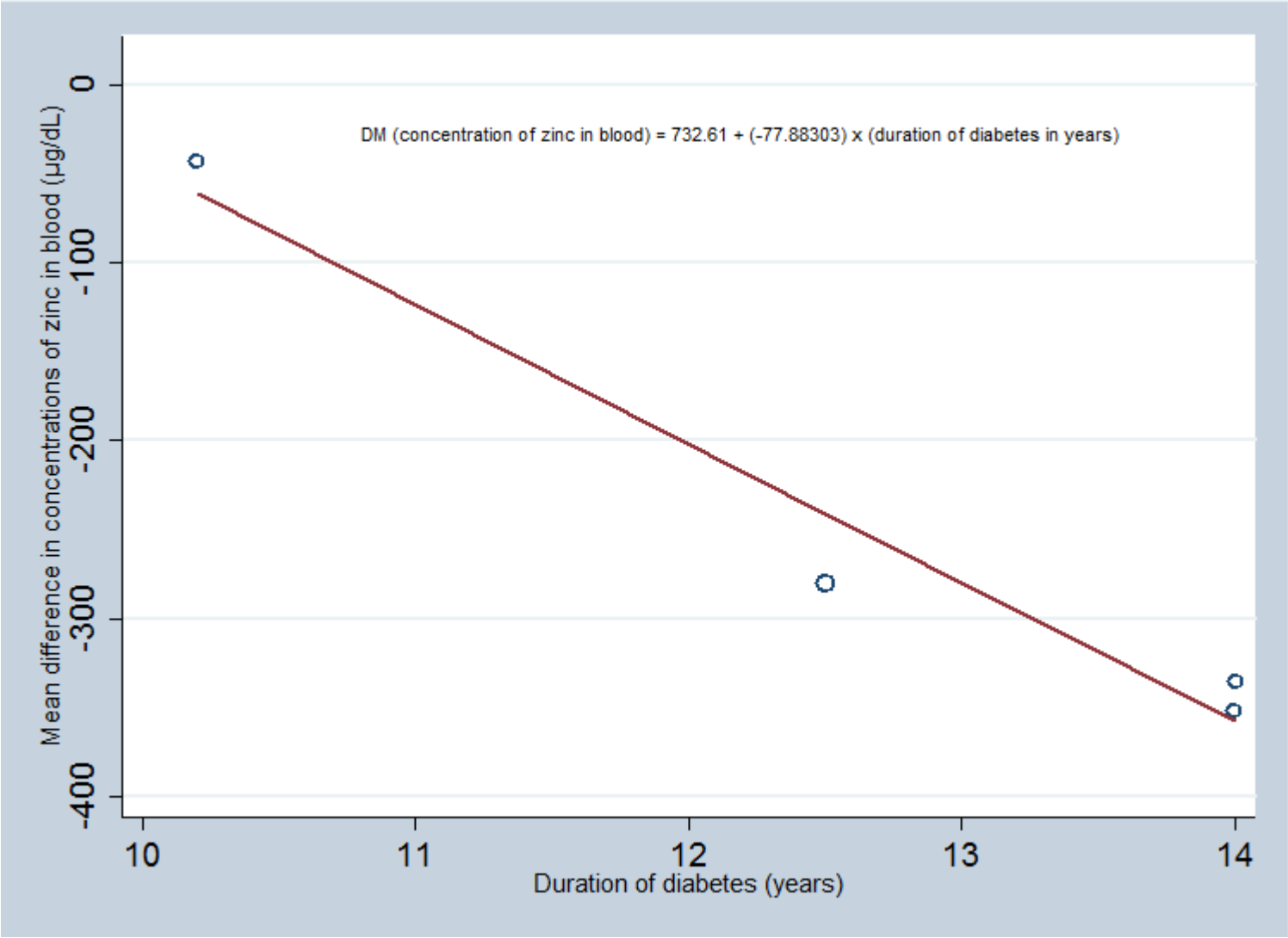
Age of non-diabetics 50 (years)									
<50	1	-280.00 (-320.73; -239.27)	-	-					
≥50	4	-178.44 (-369.42; 12.55)	98.4%	<0.001	101.57 (-580.72; 783.86)	0.668	36305	98.43%	-24.55%
No data	1	-358.00 (-617.29; -98.71)	-	-					
Older group II									
Same age (1)	4	-320.83 (-361.46; -280.19)	47.1%	0.129	-309.20 (-408.99; -209.42)	0.001	1012	55.19%	96.24%
Different age (2)	2	-12.41 (-70.89; 46.08)	69.3%	0.071					
Duration of diabetes (years)									
<13	2	-162.30 (-393.83; 69.24)	98.1%	<0.001	-180.84 (-692.57; 330.88)	0.268	13550	96.19%	31.12%
≥13	2	-343.86 (-379.36; -308.37)	0.0%	0.649					
No data	2	-148.21 (-512.01; 215.58)	87.2%	0.005	-	-	-	-	-
Sample size									
<150	2	-12.41 (-70.89; 46.08)	69.3%	0.071	-309.20 (-408.99; -209.42)	0.001	1012	55.19%	96.24%
≥150	4	-320.83 (-361.46; -280.19)	47.1%	0.129					
Matched									
No matched	2	-12.41 (-70.89; 46.08)	69.3%	0.071	-309.20 (-408.99; -209.42)	0.001	1012	55.19%	96.24%
Matched by age, gender & weight	4	-320.83 (-361.46; -280.19)	47.1%	0.129					
Zinc assessment method									
ASS	4	-237.46 (-411.56; -63.35)	98.3%	<0.001	69.65 (-389.57; 528.86)	0.695	31017	97.80%	-15.21%
ICP	2	-174.03 (-477.49; 129.42)	81.6%	0.020					

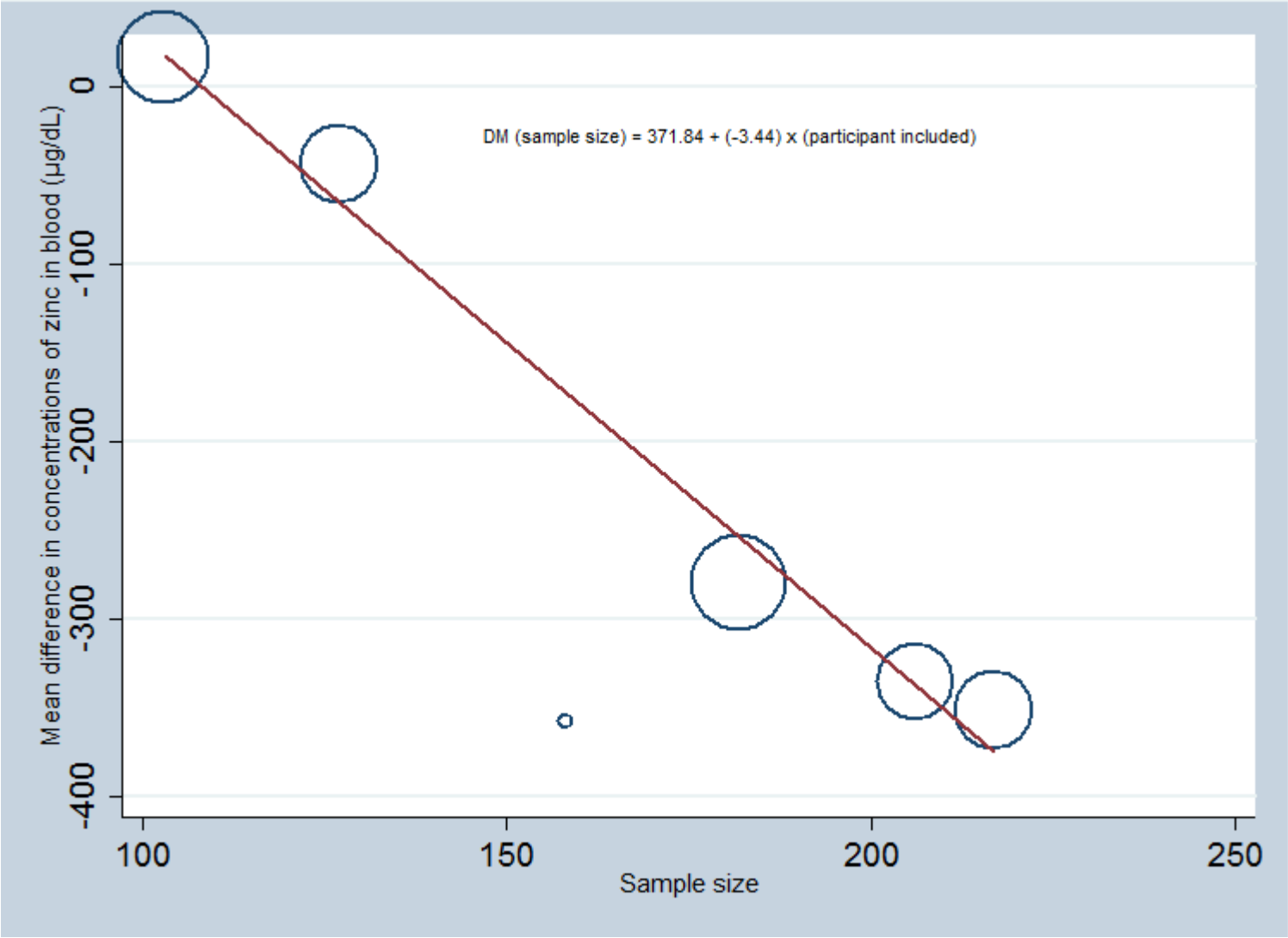
AAS (Atomic Absorption Spectrophotometry), ICP (Inductively coupled plasma), EAAS (Electrothermal Atomic Absorption Spectroscopy).


Study
ID
ES (95% CI)
%
Weight
Without complications of diabetes
Rauscher, 1997 (United Kingdom, Europe) women
0.80 (-1.90, 3.50)
10.21
Rauscher, 1997 (United Kingdom, Europe) men
2.10 (-0.46, 4.66)
10.80
Al-Maroof, 2006 (Iraq, Asia) men / women
-0.20 (-1.37, 0.97)
17.91
de Sousa Lima, 2011 (Brazil, America) men
2.00 (-3.77, 7.77)
3.54
de Sousa Lima, 2011 (Brazil, America) women
0.40 (-1.50, 2.30)
13.93
Foster, 2012 (Australia, Oceania) women
1.20 (-1.66, 4.06)
9.60
Subtotal (I-squared = 0.0%, p = 0.640)
0.41 (-0.41, 1.24)
65.98
With complications of diabetes
Lee, 2005 (Korea, Asia) women
-1.77 (-2.83, -0.71)
18.49
Batista, 2006 (Sao Paulo, America) women
-3.50 (-6.71, -0.29)
8.38
Batista, 2006 (Sao Paulo, America) men
-4.10 (-7.73, -0.47)
7.15
Subtotal (I-squared = 11.9%, p = 0.321)
-2.26 (-3.49, -1.02)
34.02
Overall (I-squared = 61.0%, p = 0.009)
-0.40 (-1.59, 0.79)
100.00
NOTE: Weights are from random effects analysis
-4
0
8


DM (dietary zinc intake) = 1.67 + (-0.37) x (duration of diabetes in years)
Mean difference in dietary zinc (mg/day)
2
0
-2
-4
5
10
15
Duration of diabetes (years)


Study
ID
ES (95% CI)
%
Weight
Western
Ekmekcioglu, 2001 (Austria, Europe) 16.01 (-26.10, 58.13) 17.80
Forte, 2013 (Italy, Europe) women/men -43.73 (-93.14, 5.67) 17.70
Subtotal (I-squared = 69.3%, p = 0.071) -12.41 (-70.89, 46.08) 35.50
Eastern
Kazi, 2008 (Pakistan, Asia) men -351.80 (-401.08, -302.52) 17.70
Kazi, 2008 (Pakistan, Asia) women -335.30 (-386.48, -284.12) 17.67
Afridi, 2009 (Pakistan, Asia) women -280.00 (-320.73, -239.27) 17.82
Chen, 2012 (China, Asia) -358.00 (-617.29, -98.71) 11.32
Subtotal (I-squared = 47.1%, p = 0.129) -320.83 (-361.46, -280.19) 64.50
Overall (I-squared = 97.7%, p = 0.000) -216.80 (-359.42, -74.19) 100.00
NOTE: Weights are from random effects analysis
-620
0
60


DM (concentration of zinc in blood) = 732.61 + (-77.88303) x (duration of diabetes in years)
Mean difference in concentrations of zinc in blood (µg/dL)
0
-100
-200
-300
-400
10
11
12
13
14
Duration of diabetes (years)


DM (sample size) = 371.84 + (-3.44) x (participant included)
Mean difference in concentrations of zinc in blood (µg/dL)
0
-100
-200
-300
-400
100
150
200
250
Sample size