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Agh F, Hasani M, Khazdouz M, Amiri F, Heshmati J, Aryaeian N. The Effect of Zinc Supplementation on Circulating Levels of Brain-Derived Neurotrophic Factor (BDNF): A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Int J Prev Med. 2022 Sep 20;13:117.

What We Know, Think We Know, or Are Starting to Know

That zinc plays a crucial role in human nutrition was first established in the early 1960's, when a series of case reports related deficiency in this important mineral to stunted growth and sexual maturation in both male and female adolescents [(1)]. It turned out that zinc is kind of a big deal. The issue with this big deal is that we don't *really* know as much as we would like given the dizzying array of physiological functions that require zinc.

Zinc appears to be highly flexible in its ability to exert diverse biological functions, and over 300 enzymes are zinc-dependent [(2)]. "Metalloproteins" are proteins in the body that bind to metal minerals [e.g., iron, copper, zinc], and up to 10% of the human genome encodes for zinc-containing proteins [(3)].

The human body contains ~2-3g of zinc, and zinc homeostasis is complex [(2,4)] [see ***Geek Box** below for further detail]. Both zinc in cells [intracellular] and "free" zinc in the circulation [extracellular] are maintained in tight ranges, and zinc levels in the body are based on zinc requirements [i.e., greater absorption when lower levels are detected] [(2,4,5)].

In the brain, around 10% of the zinc is free zinc and is highly concentrated in the hippocampus and amygdala, and zinc is essential to the development of the central nervous system [(6,7)]. However, zinc in the brain may be a Janus-faced compound; inadequate zinc may impair brain function, while excess zinc may contribute to neurodegenerative disease risk [(6–8)].

In what circumstances might zinc be beneficial? There is evidence that zinc levels may be lower in individuals with depression [(9)], and that supplementation may improve depressive symptoms [(10)]. One proposed explanation is that zinc supplementation may enhance levels of brain-derived neurotropic factor [BDNF], which is crucial for cognition [(11)]. The present study investigated the effects of zinc supplementation on BDNF levels.

*Geek Box: Mechanisms of Zinc Homeostasis

Zinc homeostasis is controlled by several factors: zinc transporters [ZTs], zinc importers [ZIPs], and metallothionein's [MTs], which are zinc-sensing molecules in the cell that regulate intracellular zinc levels. Zinc is absorbed by ZIPs in the small intestine, and zinc levels are based on physiological need and regulated at the level of absorption. For example, when levels of zinc in intestinal cells is high, concentrations of free zinc are limited in order to maintain homeostasis.

Intracellular zinc is tightly regulated to prevent the adverse effects of either zinc deficiency or zinc excess. The expression of ZTs, ZIPs, and MTs, is regulated by zinc levels, and has been shown to be tissue specific, i.e., different responses to zinc levels in different tissues.

When free zinc levels in the cell become elevated, MTs bind to that free zinc in order to maintain free zinc concentrations within tight ranges. Zinc levels in the cell are also maintained by a process of transferring zinc within the cell [i.e., from the cytosol to organelles], or exporting zinc out of the cell. All of these mechanisms are designed to respond to fluctuations in free zinc levels within the cell, and maintain zinc homeostasis.

The Study

The investigators conducted a systematic review and meta-analysis of intervention trials investigating the effects of zinc supplementation on BDNF. To be included, the primary studies had to meet the following criteria:

- **Design**: Randomised controlled trials [RCTs] of zinc as a single treatment.
- **Intervention/Exposure**: Zinc supplementation [no restrictions on supplement type or dose].
- **Control**: A placebo comparison to the zinc supplementation group.
- **Duration**: None specified.
- **Outcome**: Primary outcome was BDNF levels comparing the zinc supplementation to placebo/control groups. Secondary outcome was changes in serum zinc levels.

The outcomes were expressed as standardised mean difference [SMD; see ***Geek Box** below for further detail] and 95% confidence intervals [95% CI].

Subgroup analyses were conducted to investigate the influence of the following factors on the outcomes: study duration, supplement dose, and study quality.

*Geek Box: Standardised Mean Difference

In a meta-analysis, it is common to see "SMD" as the outcome measure. And it is actually important to distinguish between "mean difference" [MD] and "standardised mean difference" [SMD]. For MD, the outcomes are expressed in the unit of measurement, e.g., bodyweight. This assumes all studies have used the same outcome measure.

For example, suppose we are looking at a meta-analysis of 10 studies on the effects of meal timing on blood glucose levels, and all studies have measured plasma glucose response in mg/dL or mmol/L. Because one can easily be converted into the other, the researchers could decide to use MD expressed as mmol/L. Thus, if the outcome was a difference of 0.6 [95% CI 0.2 – 0.9], you would interpret this as 0.6mmol/L with a confidence interval range of 0.2mmol/L to 0.9mmol/L.

Now, suppose the analysis wanted to look at insulin sensitivity, and of our 10 studies, 4 had used HOMA-IR, 4 had used hyperinsulinemic/euglycemic clamp, and 2 had used the Matsuda Index. These are all different outcome measures for the same conceptual outcome, i.e., insulin sensitivity. This is when SMD is used, where the included studies have measured the exposure and outcome using different methods.

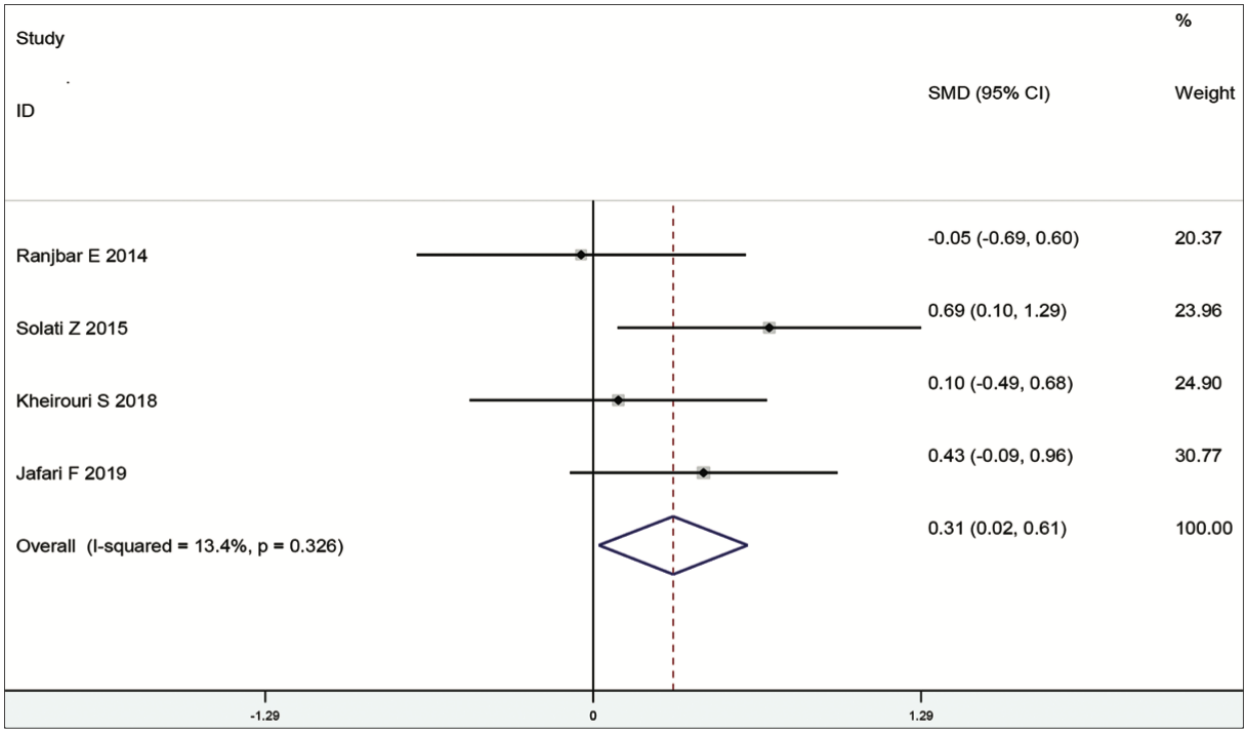
SMD is calculated by taking the mean difference from each study and dividing it by the standard deviation in that study. By doing this for each study, the SMD for each study may be combined in a meta-analysis. However, it is crucial to correctly interpreting an outcome expressed as SMD that, unlike MD, SMD is not expressed in the unit of measurement.

Rather, SMD is a measure of effect size, which is also referred to as Cohen's d after the statistician who proposed these measures. As a general rule, effect sizes of 0.2, 0.6, and 0.8, are considered small, medium, and large effect sizes, respectively. So, if the outcome now was an SMD of 0.6 [95% CI 0.2 – 0.9], you would interpret this as a medium effect size with a confidence interval range of small to large.

Results: Four RCTs were included in the study. The total number of participants from all included studies was 185; *n* = 92 and *n* = 93 from intervention and control groups, respectively. Of the four included RCTs, three reported on the secondary outcome of serum zinc levels. Three trials used zinc gluconate at 30mg/d, while one trial used zinc sulphate at 25mg/d.

***Primary Outcome – Effect of Zinc Supplementation on BDNF*:** The overall effect of zinc supplementation from all four included RCTs was an increase in BDNF levels compared to controls, with an SMD of 0.31 [95% CI 0.22 to 0.61]. Thus, the overall effect size for zinc supplementation was small, and the confidence intervals ranged from small-moderate effect sizes.

Subgroup analysis indicated that zinc doses of 30mg/d increased BDNF levels with a small-moderate effect size [SMD 0.41, 95% CI 0.08 to 0.61], but that doses of 25mg/d had no effect [discussed further under ***Interesting Finding,*** below].



Forest plot *from the paper illustrating the overall effect of zinc supplementation on BDNF levels. Note that the results are presented as SMD (95% CI), which you can see marking the top of the column second from right. You can see the effect size estimate [the square] and confidence intervals [the line] for each individual study, and the numerical presentation of the SMD and 95% CI. The trial at the top of the plot [Ranjbar E., 2014] was a trial in participants with major depressive disorder, using a dose and form of zinc of 25mg/d zinc sulphate.*

***Secondary Outcome – Effect of Zinc Supplementation on Serum Zinc Levels*:** Based on three included studies which also assessed changes in serum zinc levels, zinc supplementation increased serum zinc levels with an SMD of 0.88 [95% CI, 0.54 to 1.22]. Thus, zinc supplementation increased serum levels with a large effect size, and confidence intervals range from medium to very large.

The Critical Breakdown

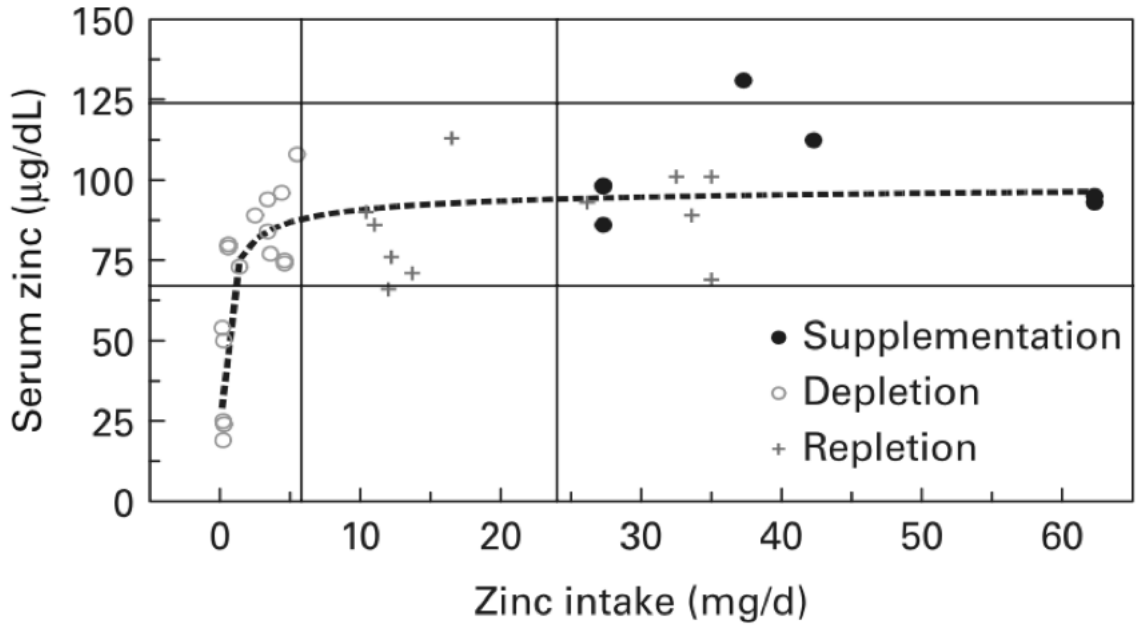
Pros: The study was pre-registered with PROSPERO [the National Institute for Health Research database for systematic reviews], and the inclusion criteria was sufficiently defined. All included trials were conducted as double-blind RCTs with similar zinc supplements and doses, and intervention duration. The intervention and control groups were balanced for numbers of participants. There was very low heterogeneity between the included studies, reflecting their relatively similar design, duration, etc. The results were clearly presented.

Cons: The meta-analysis includes only a handful of studies, with very small sample sizes in each study and a small overall sample size in the meta-analysis. The included studies, although similar in numerous design elements, were each in very different patient populations; major depressive disorder, overweight/obese but otherwise healthy, non-proliferative diabetic retinopathy, and young women with premenstrual syndrome, which may have influenced BDNF levels. For example, baseline BDNF levels were substantially lower in participants with major depressive disorder compared to baseline status in the other included trials.

Key Characteristic

Perhaps the most important characteristic of the present study is the consideration of the effects of zinc supplementation on serum zinc levels. This warrants some discussion, because establishing a valid biomarker for zinc has been challenging, and currently plasma/serum measures of zinc are the only accepted biomarker of zinc status [(12)], but with "many limitations and constraints", primarily the fact that it represents <0.2% of total body zinc [(13)].

Serum zinc concentrations are maintained within a narrow range 78 to 98mg/dL. The **figure** below illustrates this dose-response curve nicely; plasma zinc levels drop sharply <2-3mg/d zinc intake, but once intake reaches ~25mg/d there is a plateau in serum zinc response [(12)].



Response to zinc supplementation depends on baseline zinc status, with those with low or moderate baseline zinc levels exhibiting the most pronounced response to zinc supplementation, while individuals with high baseline zinc status do not respond to supplementation [12].

Further, because of the tight homeostatic regulation of zinc concentrations in the body, short-term rises in serum zinc such as those observed over 12-weeks of supplementation in the present meta-analysis may not be sustained over longer periods [13].

In some of the included studies in the present meta-analysis, serum zinc was bumped above the reference range, although this would likely be transient as the mechanisms regulating zinc homeostasis adapted [i.e., increasing zinc excretion to return levels within normal ranges] [13]. In the Jafari *et al*. paper, baseline zinc levels were 10ug/dL higher in the placebo group at baseline, thus the SMD appears inflated in favour of the change in the intervention group.

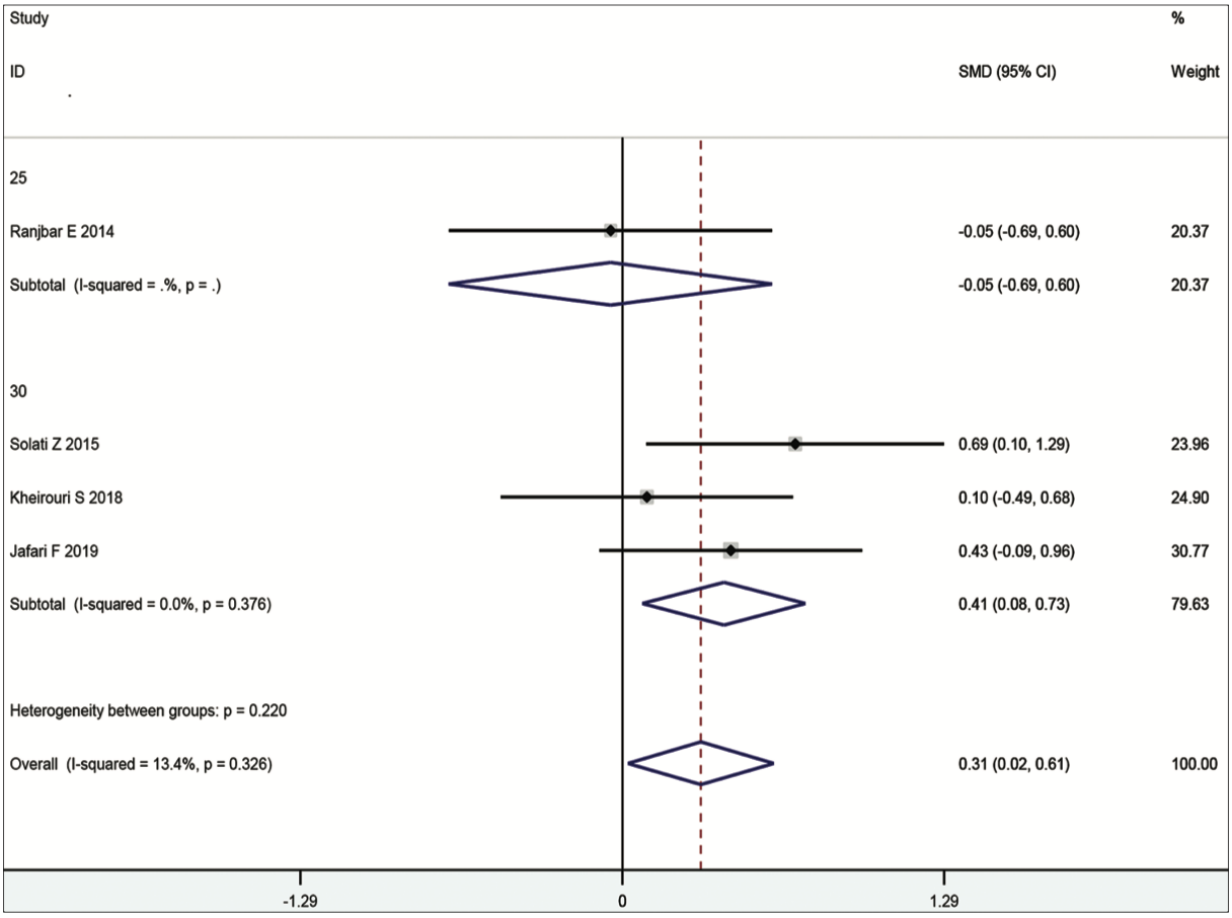
Overall, I would be cautious with interpreting the relevance of this outcome from the present study. Plasma/serum zinc is primarily considered a biomarker for population-level zinc analyses, rather than individual-level, because at an individual-level, changes in serum zinc status are not strong predictors of functional outcomes, e.g., growth [12].

Interesting Finding

Based on the discussion under ***Key Characteristic***, above, it is interesting that subgroup analysis for effects of zinc supplementation on BDNF levels only appeared to consider the supplement dose, given the difference was only ~5mg/d.

However, the one trial in that analysis showing a lack of effect of zinc supplementation on BDNF [Ranjbar E, 2014; see **figure** below] also used a different form of supplement [zinc sulphate vs. gluconate in the other trials], and was in a population with major depressive disorder. BDNF levels also increased in the placebo group to an equal degree, so there are potential issues with a lack of effective control in this trial.

This subgroup analysis would have produced the same outcome irrespective of whether they stated it was subgroup by dose or by supplement type, because the differentiation of the primary studies would have been the same.



Thus, the authors overlook that the difference in this subgroup analysis may not be related to supplemental zinc dose, and may relate to type of zinc supplement used, population characteristics, and potential issues with the control in the primary study. Meta-analysis can be misleading if we're not paying attention!

Relevance

If there is a cautious tone to this Deepdive, with an emphasis on zinc homeostasis, serum zinc ranges, and the utility of serum zinc as a biomarker, it is because there remain gaps in our understanding of zinc that warrant caution against unnecessary supplement use.

But let's think about this caveat in more detail, starting with the primary outcome of BDNF levels. The important role of BDNF in brain function is well-established, with BDNF influencing neurogenesis [i.e., the growth and survival of brain cells] and synaptic plasticity [i.e., the ability of synapses to communicate and develop new communication patterns over time] [(14)].

Less conclusive, however, is the role of zinc as a factor increasing BDNF levels. Another recent meta-analysis of five RCTs of zinc supplementation and BDNF, which included all of the four studies included in the present analysis, found a similar overall effect size of 0.31, but with 95% CI range from -0.08 to 0.67 [(15)].

While the direction of effect size and overall direction of effect is similar to the present study, it demonstrates that the addition of just one more study can change the significance of the findings. This implies caution in over-extrapolating the findings from a pool of very small, short-term studies producing small effect sizes.

Now consider that the effect size for exercise on BDNF in healthy individuals is 0.53 [95% CI 0.31 to 0.75] [(16)], a medium effect size where the lower bound of the confidence intervals reflects the overall effect size of zinc supplementation. In individuals with neurodegenerative conditions, the overall effect size for exercise on BDNF levels is a whopping 2.22 [95% CI 1.33 to 3.12] [(17)].

Consider also that the evidence for potential benefits of zinc supplementation for depression is similarly weak; in a recent systematic review of four trials, only two were in participants with diagnosed depression, and the interventions also involved additional micronutrient/multivitamin supplementation [(10)].

Application to Practice

In sum, if increasing BDNF levels is a desired outcome for cognitive function and health, there are more effective ways to achieve this end, in particular exercise [(16,17)]. While zinc is important for central nervous system development and function, it should also be noted that two brain regions in which there are high zinc concentrations – the hippocampus and amygdala – are also two regions affected by plaque formation and neurodegeneration in Alzheimer's Disease [(7)].

Both human and animal studies show high concentrations of zinc in amyloid plaque, suggesting zinc may promote beta-amyloid plaque development, which may be due to overactivity of zinc transporters leading to higher concentrations of brain zinc [(6)].

The evidence to suggest zinc as an intervention for brain health is insufficient at this point. There is, generally, little evidence to suggest a need for supplementation in otherwise healthy individuals, except for individuals who have an inadequate intake of dietary zinc. In that context, proceed with caution: there appears to be little reason to supplement beyond 25-30mg/d zinc.

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