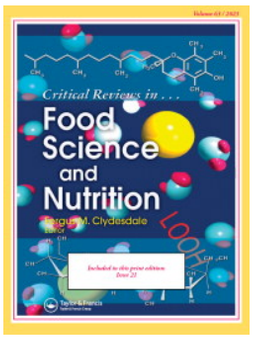

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SYSTEMATIC REVIEW

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FADS1 and FADS2 as biomarkers of Zn status – a systematic review and meta-analysis

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ABSTRACT

Despite enormous research efforts, a sufficiently sensitive and reliable biomarker for the assessment of zinc (Zn) status has not been identified to date. Zn affects fatty acid metabolism and alters the activity of certain desaturases; thus, desaturase activity has been proposed as a potential new biomarker of Zn status. This systematic review complied and assessed studies that examined changes in fatty acid desaturase 1 (FADS1) and fatty acid desaturase 2 (FADS2) activities in relation to modifications in dietary Zn intake. A systematic search was performed in PubMed, Web of Science, Scopus, Web of Knowledge, and Central with strictly defined search, inclusion, and exclusion criteria. Twenty-one studies were included, 8 animal and 13 human trials (5 randomized controlled trials, two non-randomized controlled trials, and 6 cross-sectional studies). This systematic review was performed using PRISMA guidelines and where feasible a random-effects meta-analysis was conducted. No significant correlation was seen between the delta 6 desaturase and Zn status (-0.0958, 95% CIs (-0.2912; 0.1074), p = 0.2928). Delta 6 desaturase seems to respond in a greater magnitude than Zn status to Zn-containing interventions (the standardized mean difference for delta 6 desaturase was −0.6052, 95% CIs (-2.7162; 1.5058), p = 0.4289, while for plasma/serum Zn it was 0.0319, 95% CIs (-0.9133; 0.9770), p = 0.9213). Finally, two separate meta-analyses on same studies that assessed the correlations between LA:DGLA and Zn intake and Zn status and Zn intake revealed that the magnitude of correlations was only slightly different (the pooled correlation coefficient between the LA:DGLA ratio and Zn intake had a value of −0.1050, 95% CIs (-0.5356; 0.3690), p = 0.454, while between plasma Zn and Zn intake had a value of −0.0647, 95% CIs (-0.4224; 0.3106), p = 0.5453). According to the descriptive analysis, the magnitude of variation in desaturase activities in response to Zn intake was not consistent among studies, FADS1 and FADS2 activity corresponded to dietary Zn manipulations, both in animals and humans. A plausible explanation for this observation might be the difference between the studies in study populations, types of dietary interventions, study durations, etc. In addition, several potential confounders and covariates are identified from the qualitative synthesis, such as gender, age, the type of fat provided within the dietary intervention, the size of Zn particles, among others. Further high-quality studies are needed to additionally clarify the suggested associations and applicability of utilizing fatty acid desaturase activities as Zn status biomarkers.

KEYWORDS

Zinc; Zn; biomarker; desaturase; LA:DGLA ratio; zinc interventions; zinc deficiency

Introduction

Zinc (Zn) deficiency is estimated to affect 17% of the global population (Wessells and Brown, 2012).

It accounts for up to 4% of global child and infant mortality, with the largest number of people affected living in developing countries, Sub-Saharan Africa, and South Asia (Kumssa et al. 2015). In developed countries, severe Zn deficiency is uncommon, still marginal deficiency of Zn is much more widespread (Gibson et al. 2008; Kumssa et al. 2015). The identification of marginal Zn deficiency is challenging due to the lack of a specific and reliable biomarker of Zn status.

Plasma or serum Zn is currently the most commonly used biomarker for assessing Zn status, and while convenient for populational studies, it is a poor indicator of Zn status on an individual level (Lowe 2016; King et al. 2015). Zn homeostasis is tightly regulated, so circulating concentrations of Zn often stay unaffected by dietary Zn modifications. In addition, plasma Zn is influenced by factors not related to Zn status, i.e., inflammation, infection, pregnancy, medication (Prasad et al. 2007; King et al. 2015).

Therefore, serum/plasma Zn levels within the reference ranges do not necessary imply the absence of Zn deficiency. Hence, a more sensitive and more precise biomarker is

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required to offer a more objective evaluation of Zn status and detect early-stage Zn deficiency states.

Over the recent years several new indicators have been proposed as potential candidates for more accurate determination of Zn status, one of them being the activities of fatty acid desaturases 1 and 2 (FADS1 and FADS2). Adequate Zn intake is necessary to support omega-6 fatty acid metabolism (Arnold, Pinkham, and Votolato 2000). FADS1 and FADS2 are Zn-dependent enzymes essential for metabolizing linoleic acid to arachidonic acid (Horrobin and Cunnane 1980; Ayala and Brenner 1983). Fatty acid desaturase activities in relation to Zn intake and status have been examined both by experimental animal models and clinical trials, and direct associations between low dietary Zn intake and FADS1 (delta 5 desaturase) and FADS2 (delta 6 desaturase) activities are noticed (Clejan et al. 1982; Cunnane, Horrobin, and Manku 1984; Cunnane and Krieger 1988; Ayala and Brenner 1983; Eder and Kirchgessner 1995).

In addition, several studies have demonstrated that Zn deficiency affects the ratio of two essential omega 6 amino acids, linoleic (LA) and dihomo-gamma-linolenic acid (DGLA) and proposed that the LA to DGLA ratio could be used as a novel biomarker of Zn status, both in animals and humans (Reed et al. 2014; Knez et al. 2016; Knez et al. 2018; Chimhashu et al. 2018). However, additional research is required to demonstrate the full potential of this promising biomarker. Further research is needed to identify and examine potentially more effective biomarkers of Zn status with improved sensitivity and specificity able to respond to modest changes in dietary Zn intakes in humans.

The objective of the current work was to conduct a systematic review of experimental animal and human studies on the efficacy of FADS1 and FADS2 to respond to Zn interventions and to define the current applicability and suitability of FADS activities to act as biomarkers of Zn status in humans.

Materials and methods

Review protocol and registration

The systematic review was registered in Prospero (ID: CRD42021283533) under the following research question "Diagnostic performance of FADS1 and FADS2 for assessment of Zn interventions, a systematic review". Up to our knowledge this is the first systematic review on this topic. The Preferred Reporting Items for Systematic review and Meta-Analysis (PRISMA) protocols were applied (Moher et al. 2009).

Literature search strategy

Systematic searches were completed using several databases, including PubMed, ISI (Web of Science), Scopus, ISI (Web of knowledge), and CENTRAL (Cochrane Central Register of Controlled Trials).

Two researchers (MK and AP) identified original articles independently. In addition, a manual search of the reference list of the included articles was done to identify other potentially relevant articles.

Finally, clinical trial registries were examined (https://clinicaltrials.gov/ and https://www.clinicaltrialsregister.eu/), with the keywords focusing on fatty acid ratios, to identify registered ongoing trials.

The search criteria were developed by combining a set of keywords with Boolean operators, aiming to define exposure (intervention - zinc) and outcomes of interest (fatty acid ratios and nervonic acid).

The complete search strategy is presented the Supplementary file.

Inclusion and exclusion criteria

As this systematic review intended to comprehensively summarize all reported data related to the relationship between Zn and fatty acid ratios, broader inclusion criteria were applied.

Therefore, for the qualitative synthesis, in vitro, animal, and human studies, reporting either an association between Zn status or Zn-related fatty acid ratios, including nervonic acid, or the effect of a Zn-containing intervention on the levels/activity of certain predefined fatty acid ratios were included. No restrictions were applied regarding the study models included. All relevant articles published between January 1963 to January 2022 were included.

Studies that were not published in English, without full texts, not reporting the outcome of interest, and studies using an intervention that included Zn combined with some other bioactive components were excluded.

Study selection

All identified articles were downloaded from electronic databases into the EndNoteX9 software and duplicates were removed. The final list of articles was uploaded to Rayyan software (Ouzzani et al. 2016) for further screening processes. Titles and abstracts were reviewed independently by two researchers following previously formulated inclusion and exclusion criteria. Discrepancies were resolved by mutual conversation and via consensus with a third reviewer as required.

The full texts of the selected articles were downloaded and reviewed according to the defined criteria in the same manner, data were compared to ensure consistency and inconsistent judgments were resolved by consensus.

Data extraction

All relevant information from included studies were extracted to an Excel sheet and later presented in tables and figures. The table created for human studies data contained the following information: 1) name of the first author, 2) country in which the study was conducted, 3) information about study design, 4) brief description of the study population (i.e. percentage of women, mean/median age, health status of the study population), 5) total number of subjects or the

number of subjects assigned to the intervention/control arm (where applicable), 6) description of the intervention (where applicable), 7) duration of the intervention (where applicable), 8) main findings (primary outcomes) - related to LA:DGLA ratio, desaturase 5 and 6, elongase and nervonic acid 9) secondary outcomes - plasma/serum levels and dietary intake of Zn, and information on how these assessments were performed.

The table presenting animal studies included similar information: 1) name of the first author, 2) country in which the study was conducted, 3) study design, 4) brief description of the studied animals 5) description of study groups, 6) study duration 7) information on samples used to analyze the outcomes of interest, 8) main findings (primary outcomes) - related to LA:DGLA ratio, desaturase 5 and 6, elongases and nervonic acid and information on how the desaturases/elongases were estimated, and 9) secondary outcomes - plasma, dietary intake of Zn and methods of assessment.

Risk of bias assessment

The methodological quality of RCTs was evaluated with a revised risk of bias tool (RoB2 tool) for parallel and cross-over designed studies developed by the Cochrane group (Sterne et al. 2019). The RoB2 tool was used for assessing the biases arising at different stages of a trial (known as the bias domains), including the following: bias arising from the randomization process, biases arising from deviations from intended interventions (described as intervention assignments and adherence effects), missing outcome data, due to outcome assessment and selective outcome reporting. Each study was classified as having a 'high', 'low' risk of bias or 'some concerns', following a predefined algorithm (Supplementary Table 1).

The risk of bias of non-randomized studies was evaluated using the Newcastle Ottawa Scale (NOS), adapted for cross-sectional studies (Modesti et al. 2016). The scale uses a star system, where a study could have a maximum of 10 stars, after being evaluated for 3 main domains: "selection"(a maximum of 5 stars) - this domain evaluates the appropriateness of the study design, recruitment of participants and response rate); "comparability"(a maximum of 2 stars) - this domain assesses comparability between different outcome groups and controls for the most important confounders; however, since the studies included generally had only one group, we considered whether the study controlled for the most important confounders in the inclusion/exclusion criteria; and "outcome" (maximum 3 stars - assessing the suitability of applied statistical tests (Supplementary Table 2).

The risk of bias in animal studies was evaluated using SYRCLE's risk of bias tool, which is based on the Cochrane RoB tool and adjusted for animal studies (Hooijmans et al. 2014). This tool assessed the bias that could arise from 6 different domains: selection (referring to randomization process), performance (reflecting whether staff blinding was performed, and whether animals were housed randomly), detection (specifying whether outcome assessment was performed in a random and blinded manner), attrition (caused by incomplete outcome data), reporting (caused by selective outcome reporting), and bias caused by some other causes (Supplementary Table 3).

Meta-analysis

As included studies were performed on diverse populations and different types of interventions and Zn dosages were used, the random-effects model meta-analysis was applied to accommodate for heterogeneity. After conducting the qualitative synthesis, studies that assessed the same outcomes were identified. Thus, two types of analyses were carried out – the first one focused on pooling the correlation coefficients from studies that reported the correlations between the LA:DGLA ratio and Zn status and intake. This was done with the *metacor* function which uses the generic inverse variance pooling method and is based on Fisher z-transformation of correlations. In this case, it was not necessary to perform any pre-processing of the data, as all studies reported the outcomes of interest homogeneously and could be included in the analysis.

The other type of analysis focused on pooling the weighted and standardized mean differences between the intervention and control groups by using the *metacont* function, which also relies on the generic inverse variance method to pool the data. To enhance the homogeneity of the studies, FADS1 activity was expressed as arachidonic acid shown as AA/DGLA, not as ARA/DGLA as it was originally reported in the article by Lowe et al. (2021).

In addition, the FADS1 and FADS2 indices originally reported in the study conducted by Jongstra et al. 2022 (as geo-mean (range), were re-calculated into arithmetic mean (standard deviation). The endpoints of FADS1 activity described by Hernandez et al. 2020 as geo-mean (95%CIs) were converted into mean (SD) using the method suggested by Higgins, White, and Anzures-Cabrera 2008). FADS2 activity reported as median (interquartile range) was changed into mean (SD) according to the method provided by McGrath et al. (2020).

The re-calculation of the raw data was accomplished when the authors provided the datasets upon our request ($n = 2$ out of 8 datasets in total). In both analyses, Hartung-Knapp adjustment for the random-effect model was applied, and the restricted maximum-likelihood estimator for estimating the between-study variance.

Between-study heterogeneity was assessed with Cochrane's Q and I^2 tests (Higgins et al. 2003), and $I^2 > 50\%$, and $p < 0.05$ indicated significant heterogeneity. Less than 10 studies were included in this meta-analysis therefore the assessment of publication bias was not performed.

Results

Study selection process

The flow diagram of the literature search process was created based on PRISMA (the Preferred Reporting Items for Systematic reviews and Meta-Analysis) guidelines (Figure 1).

Table 1. Summary of characteristics and findings of RTCs and Zn-controlled feeding trials.

Reference	Study design / Country	Study population	Type and content of Zn intervention	Control arm	Duration	D5D outcomes	D6D outcomes	Estimation of desaturases
Lowe et al. 2021	RCT (cross-over design) / Pakistan	Women of reproductive age from 50 households, n = 25 randomized to bio-fortified wheat and n = 25 randomized to control flour	Zinc bio-fortified wheat (Zincol-2016) – 49.3 ± 5.6 mg/kg)	Control flour (Galaxy) – 22.2 ± 2.9 mg. kg	2-week baseline period with the control flour, 8-week intervention period -received the bio-fortified flour or the control flour	Group A - no significant difference in FADS1 activity index. Group B – marginally significant increase after 8 weeks of intervention (p = 0.059).	Group A – no significant difference in FADS2 activity. Group B – significant decrease after 8 weeks of intervention.	FADS1 = ARA/DGLA FADS2 = GLA/LA
Jongstra et al. 2022	RCT / Bangladesh	520, 12-36 months old children, zinc bio-fortified group n = 259, control group n = 261	Zinc bio-fortified rice in cooked portions to their households daily (28.5 ± 3.5 µg/g of Zn in raw rice)	Control rice in cooked portions to their households daily (14.7 ± 0.8 of Zn in raw rice)	9 months	No significant effect of treatment on D5D activity. The time-by-treatment interaction was not significant.	No significant effect of treatment on D6D activity. The time-by-treatment interaction was not significant either.	D5D = AA/DGLA D6D = GLA/LA
Liong et al. 2021	Controlled feeding trial / USA	36 healthy adult men, aged 18 to 51 years	Bread made from zinc-biofortified wheat (10.9 mg zinc/d) with no additional phytate (0.6 g/d total phytate) for 6 weeks and 25-mg zinc supplement for 2 weeks	Placebo capsules for 2 weeks during the last phase of trial that involved Zn supplementation	10 weeks (2 weeks of run-in period, followed by 6 weeks of bread consumption and 2 weeks of supplementation/ placebo together with the bread consumption)	FADS1 activity significantly decreased during the MP2 period, when the participants consumed 10.9 mg Zn/d (of which 6.3 mg was provided through bread without additional phytate). No changes in FADS1 activity during MP3 when 25 mg/d of supplemental Zn was given to half of the men.	FADS2 activity significantly increased during MP2 period. No change in FADS2 activity during MP3 when the 25 mg/d of supplemental zinc was given to half of the men.	FADS1 = AA/DGLA FADS2 = GLA/LA
Massih et al. 2021	RCT / USA	35 healthy men, mean age 24.7 ± 1.36 years	25 mg zinc as zinc gluconate in the fasted state 30 min before breakfast [zinc before breakfast (ZBB)]	25 mg zinc as zinc gluconate consumed with breakfast [zinc with breakfast (ZWB)]	13 days	Consumption of zinc supplement with breakfast led to a significantly different FADS1 activity index compared to consumption of supplement without food (p = 0.044).	When controlling for baseline values, FADS2 activity index with supplementation did not differ between the groups (p = 0.21).	FADS1 = ARA/DGLA FADS2 = GLA/LA
Suh et al. 2022	Before/after feeding trial / USA	Eighteen healthy men aged 19–45 y with a BMI (in kg/m2) between 18 and 30	Bio-fortified rice – 6 mg Zn/d (and 1.5 g phytate); 10-mg Zn/d diet without phytate; ad libitum diet supplemented with 25 mg Zn/d.	None	6 mg Zn/d for 2 wk (MP1) followed by a 10-mg Zn/d diet for 4 wk (MP2), and ad libitum diet supplemented with 25 mg Zn/d was then fed for 3 wk (MP3).	FADS1 activity increased significantly, by 28.9% (p = 0.007) and 45.6% (p = 0.00005) following MP1 and MP2 phases; the activity levels returned to baseline following the 25-mg Zn/d supplementation period.	FADS2 activity trended higher by 56% (p = 0.07) at the end of MP1, and it was increased by 126% (p = 0.0001) following MP2. Supplementation with 25 mg Zn/d in MP3 decreased the FADS2 activity back to baseline concentrations.	FADS1 = AA/DGLA FADS2 = LA/GLA

Table 1. (Continued).

Reference	Study design / Country	Study population	Type and content of Zn intervention	Control arm	Duration	D5D outcomes	D6D outcomes	Estimation of desaturases
Hernández et al. 2020	RCT / Chile	Sixty patients with T2D; mean age in the zinc group (n = 30) 54.9 ± 6.4; in placebo group (n = 30) 55.0 ± 7.6	30 mg of elemental zinc (as sulfate)	Placebo capsules (magnesium silicate)	24 months	There was no treatment effect for the D5D activity.	D6D activity was unaffected by Zn supplementation.	D5D n-6: AA/DGLA D6D n-6: GLA/LA
Chimhashu et al. 2018	RCT / Benin, West Africa	186 healthy children, 84 were girls; mean age in the intervention arm (n = 89): 8.10 (6.64, 9.59), in the control arm (n = 97): 8.5 (7.33, 9.58).	Water filtered with a Zn fortification chamber. Average daily zinc intake was 4·3 (IQR 3·5, 5·2) mg and 2·8 (IQR 0·0, 4·5) mg on school days and over the entire study	Water filtered with a placebo chamber	20 weeks	Correlation with Zn status: r=-0.170, p = 0.036. There was a statistically significant difference in the change of D5D activity from baseline in the placebo group (in the whole study sample, not in Zn-deficient children).	Correlation with plasma Zn: r 0.293, P ≤ 0.0001. No significant difference in the DGLA:LA ratio in the Zn-deficient group when compared with the Zn-sufficient group (0.15 (SD 0.43) and 0.14 (SD 0.42), respectively, P = 0.217)	D5D = AA/DGLA D6D = DGLA/LA

Table 2. Summary of characteristics and findings of cross-sectional studies.

Reference	Study design/ Country	Study population	D5D outcomes	D6D outcomes	LA:DGLA	Estimation of desaturases
Monteiro et al. 2021	Cross-sectional study / USA	1614 participants (771 females and 843 males) from NHANES 2011–2012 database, mean age of women: 48.6 ± 17.5; of men: 47.6 ± 17.7 years	NR	NR	Correlation with Zn status (women): r = − 0.05, p = 0.39; (men): r = − 0.11, p = 0.07. Correlations with Zn intake adjusted for energy intake in women: r=-0.05, p = 0.12, in men: r = 0.03, p = 0.32).	NA
Takic et al. 2021	Cross-sectional study / Serbia	40 adult patients undergoing hemodialysis treatment (9 women), mean age: 56 ± 15 years	Correlation with serum zinc status: r=-0.168, p = 0.296. Correlations with dietary Zn: r=-0.092, p = 0.564.	Correlations with serum zinc concentration: r = 0.311, p = 0.037. Correlations with dietary Zn: r = 0.011, p = 0.947.	Correlation with serum zinc status: r = −0.335, p = 0.033. Correlations with dietary Zn: r=-0.025, p = 0.883.	D5D: AA/DGLA D6D: DGLA/LA
Knez et al. 2018	Cross-sectional study / Serbia	27 dyslipidemic adults, median age 57.00 [16.00]	Correlations with Zn status: r = 0.168 p = 0.471.	Correlations with Zn status: r = 0.168 p = 0.443.	Correlations with Zn status: r = −0.132 p = 0.058. Correlation with Zn intake: r = −0.38, p = 0.05.	D5D: AA/DGLA D6D: GLA/AA
Knez et al. 2016	Cross-sectional study / Serbia	54 healthy volunteers, mean age: 40.4 ± 7 years	NR	NR	Correlation with Zn status: r = −0.35, p = 0.01. Correlation with Zn intake: r=-0.01, p = 0.94.	NA
Yary et al. 2017	Cross-sectional study / Eastern Finland	2189 men from the prospective Kuopio Ischemic Heart Disease Risk Factor Study, aged 42–60 y and free of T2D at baseline in 1984–1989	D5D activity was the highest in the 1st Zn status quartile, and lowest in the 4th.	D6D activity was the lowest in the first Zn status quartile and the highest in the 4th.	NR	D5D: AA/DGLA D6D: GLA/LA
Wang et al. 2008	Cross-sectional study / China	232 community-dwelling subjects aged between 35 and 60 years; mean age essential hypertension patients (n = 109): 44.3 ± 7.5; non-hypertensive patients (n = 123): 42.8 ± 4.4	NR	Correlation with Zn status: r = −0.13, p > 0.05.	NR	D6D: AA/LA

Table 3. Summary of characteristics and findings of animal model studies.

Reference	Study design / Country	Study animals	Description of study groups	Zn content
Stawarska et al. 2021	Controlled study / Poland	24 Spraque-Dawley female rats with breast cancer	3 groups, 8 rats in each group: control, and 2 groups received either Zn microparticles (342 nm) or Zn nanoparticles (99 nm).	Both micro- and nanoparticles of zinc were administered via gavage in the dose of 4.6 mg/mL in 0.4 mL of water.
Knez et al. 2018	Controlled study / USA	30 Cornish cross fertile broiler eggs *Gallus gallus*	High Zn diet n = 15, low Zn diet n = 15.	High Zn diet: 46.5 µg Zn/g; low Zn diet: 32.8 µg Zn/g
Reed et al. 2014	Controlled study / USA	48 Cornish cross-fertile broiler eggs *Gallus gallus*	2 equal groups: Zinc adequate control diet (n = 12) and Zinc deficient diet (n = 12).	Zinc adequate control diet: 42.29 µg/g zinc); Zinc deficient diet: 2.55 µg/g zinc)
Eder and Kirchgessner 1995	Controlled study / Germany	36 male Sprague-Dawley rats weighing 121 + 4 g	4 groups, 9 rats each: Zn adequate/coconut oil; Zn deficient/coconut oil; Zn adequate/linseed oil; Zn deficient/linseed oil.	The Zn deficient diet contained 0.5 mg Zn/kg. The zinc adequate diet was supplemented with 30 mg/kg Zn (as zinc sulfate-heptahydrate).
Kudo, Nakagawa, and Waku 1990	Controlled study / Japan	Three-week-old male Wistar rats weighing 50-60 g	3 groups (10-12 rats each): *ad libitum*-fed rats were allowed free access to a zinc-adequate (AL) or zinc-deficient (ZD) diet. The food restricted pair-fed (PF) rats were given the zinc-adequate diet	A zinc-adequate diet was prepared by adding zinc as Zn (CH3COO)2 ~ 2H20 to the basal diet to 50 ppm Zn. Zn deficient - <2 ppm
Cunnane and Krieger 1988	Controlled study / Canada	30 weanling male Sprague-Dawley rats (mean weight 50 (SE 2) g)	3 groups, 10 rats in each group: Zn-deficient, control and High-Zn group.	The Zn-deficient diet contained 3-4 mg Zn/kg, the control diet 36 mg Zn/kg and the High-Zn diet 411 mg Zn/kg.
Ayala and Brenner 1983	Controlled study / Argentina	12 Male weanling Wistar rats	Rats were divided into groups of 4 rats each and received either a Zn sufficient or Zn deficient diet.	55 ppm of Zn, and a Zn deficient diet (1.2 ppm)
Clejan et al. 1982	Controlled study / USA	38 male Sprague-Dawley rats, weighing 90 + 20 g	They were divided into 6 groups, out of which the 1st two were of interest: 1st group: Zn supplemented diet (n = 8), 2nd group: Zn deficient diet (n = 8).	1st group = purified Zn-supplemented diet containing 100 mg/kg Zn as Zn chloride. 2nd group = Zn-deficient diet (1.2 ppm Zn).

Study duration	Sample	D5D outcomes	D6D outcomes	Desaturase Estimation
20 weeks	Serum	The highest activity was observed in the control group, it was slightly lower in the group that received nanoparticles, and considerably lower in the group receiving Zn in microparticles.	The highest activity was observed in the control group, it was slightly lower in the group that received nanoparticles, and considerably lower in the group that received Zn in microparticles.	D5D: AA/DGLA D6D: GLA/LA
42 days	Erythrocytes	NA	There were statistically significant differences in the LA: DGLA ratio among the groups, with the higher ratio measured in the group of birds fed the low Zn diet, at all-timepoints ($p < 0.05$).	D6D: LA/DGLA
28 days	Erythrocytes	NA	The % w/w ratio was significantly elevated in the Zn-deficient group on days 7, 14, and 21 but not significantly different on day 28. Overall, the Zn-deficient group had a higher cumulative mean ratio ($p < 0.001$).	D6D: LA/DGLA
10 days (the trial had to stop as Zn def rats became severely ill)	Hepatic microsomes	Activity of D5D-desaturase was not influenced by zinc deficiency in the rats fed both types of dietary oil.	D6D-desaturase activity using linoleic acid as substrate was not different between Zn-deficient and Zn-adequate rats fed coconut oil diet but was higher in Zn-deficient rats fed linseed oil diet than in Zn-adequate rats fed linseed oil diet.	D5D: the amount of (1-14 C)-arachidonic acid produced from dihomo-7-(1-14C)-linolenic acid. D6D: the amount of (1-14 C)-linolenic acid resp. (1-14 C) octadecatetraenoic acid produced from (1-14 C)-linoleic acid resp. α-(1-14 C)-linolenic acid.
2 weeks	Total phospholipids in liver	NA	The values for D6D were higher in the group receiving Zn deficient diet *ad libitum*, compared to the group receiving Zn adequate diet *ad libitum*. The highest values were observed in rats receiving zinc-adequate diets.	D6D: AA:LA
10 weeks	Plasma and liver	NR	In the phosphatidylcholine subclass the ratio of LA/AA was higher in the Zn-deficient group compared to the controls ($P < 0.01$); in the phosphatidylserine subclass the ratio was lower in the Zn-high group compared with the controls ($P < 0.01$). In the phospholipid subclass of the liver, the ratio was higher in the Zn-deficient group than in the controls or High-Zn group.	D6D: LA/AA
60 days. After 18 days, the Zn deficient rats got ill, and were switched to the other diet until the end of 60 days.	Liver and testes tissues	Significantly higher values were presented in Zn adequate livers ($p < 0.001$) and testes ($p < 0.01$).	Higher values were presented in Zn adequate livers and testes (both $p < 0.001$).	D5D: DGLA/AA D6D: LA/GLA
6 weeks	Liver microsomes	Higher activity was measured in the Zn supplemented groups vs the Zn deficient group.	Higher activity was measured in Zn supplemented group	Desaturation reaction assays

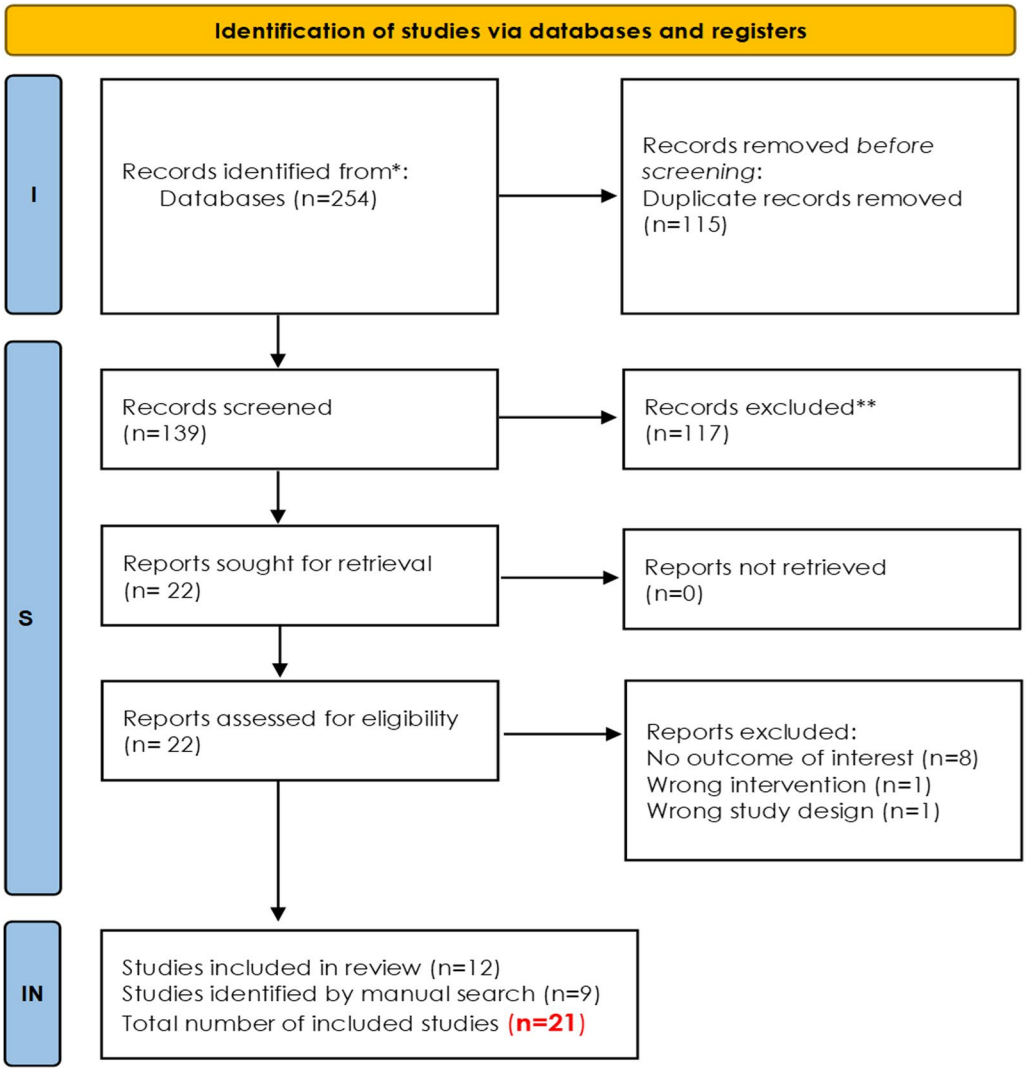

Figure 1. PRISMA Flowchart.Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.For more information, visit:http://www.prisma-statement.org/.

The systematic search retrieved a total of 254 articles: 50 from PubMed, 7 from Scopus, 41 from ISI (Web of Science), 153 from ISI (Web of Knowledge) and 3 from CENTRAL. After removing the duplicates, a total of 139 papers were uploaded to Rayyan for the screening processes and the titles and abstracts were reviewed to identify the articles that reported results related to relationship between Zn status/intake and the fatty acid ratios and nervonic acid. During this step, 117 articles were marked as irrelevant to the research question.

Thus, 22 full text articles were assessed according to predefined inclusion/exclusion criteria. Out of these 22 studies, 8 reported no outcomes of interest, 1 used the wrong intervention, and 1 had the wrong study design. Finally, we identified a total of 12 studies that could be included in this systematic review.

After manually searching the reference lists of selected articles, 5 additional studies were identified and following the authors publication checklists 4 studies were identified as being accepted for publication, so the authors were contacted via email to attain necessary information. Therefore, the final number of studies included was 21 (13 human and 8 animal model studies).

Characteristics of the included studies

The characteristics of all studies incorporated in the analysis are shown in Summary Tables 1, 2 and 3.

Five RCTs and two non-randomized controlled trials evaluated the effect of Zn supplementation on desaturase activities. All trials had a parallel design except the one conducted by Lowe and coworkers (2022) which employed the cross-over design without a wash-out period. One of the studies included 50 Pakistani households in which women of reproductive age were the study subjects (Lowe et al. 2021), one examined 520 one to three-years-old Bangladeshi children (Jongstra et al. 2022), another included 35 healthy men from the USA (Massih et al. 2021). Hernández and coworkers assessed 60 diabetic patients from Chile (Hernández et al. 2020), the last RCT included 186 children from West Africa (Chimhashu et al. 2018), while

the two non-randomized controlled trials were conducted on healthy adult men., The duration of the interventions in these trials varied from 13 days to 24 months (Massih et al. 2021; Hernández et al. 2020).

The other six studies included in the evaluation were of cross-sectional design, of which three were conducted in Serbia (Knez et al. 2016; Knez et al. 2018; Takic et al. 2021) and the other three in Eastern Finland (Yary et al. 2016), China (Wang et al. 2008) and in the USA (Monteiro et al. 2021). Number of study participants ranged from 27 to 2189 subjects and included both healthy people and patients diagnosed with a chronic condition (dyslipidemic subjects, those undergoing hemodialysis treatment, and people with hypertension). Finally, through this systematic search, eight acceptable animal model studies were identified. Three of them were conducted in the USA (Clejan et al. 1982; Reed et al. 2014; Knez et al. 2018) and one study each in Canada (Cunnane and Krieger 1988), Argentina (Ayala and Brenner 1983), Poland (Stawarska et al. 2021), Germany (Eder and Kirchgessner 1995) and Japan (Kudo, Nakagawa, and Waku 1990). The Cornell University studies used the broiler chicken (*Gallus gallus*) as the model, while others employed rats as a model system (Sprague-Dawley rats in four studies and male Wistar rats in two studies). Stawarska et al. (2021) examined rats with breast cancer, while all others included disease-free rats.

Quality of included studies

Details of the quality assessment of 5 RCTs are presented in Supplementary Table 1.

The quality of two non-randomized controlled trials was not evaluated. Three studies were considered as having a high risk of bias (Chimhashu et al. 2018; Hernández et al. 2020; Massih et al. 2021), with the bias arising from different domains. The studies conducted by Jongstra et al. (2022) and Lowe et al. (2021) were appraised as having "low risk" of bias.

The comprehensive assessment of the quality of non-randomized studies is presented in Supplementary Table 2. In general, 2 studies received the maximum number of stars, 3 were rated with 7 stars, and one with 6. Overall, the quality of the cross-sectional studies was deemed satisfactory.

Finally, the risk of bias assessments for animal studies is presented in Supplementary Table 3.

Most of the studies did not provide information of interest required based on the SYRCLE's risk of bias tool (such as these related to randomization of the animals, allocation sequence, blinding of the investigators); thus, it was impossible to draw straightforward conclusions about the quality of these studies. It should be noted that the decisions for the studies conducted by Knez et al. (2018) and Reed et al. (2014) were made based on personal communication with the authors, and the judgment for the study by Stawarska et al. (2021) was also made based on the previous work of the group. None of the studies as published provided all the necessary information for conducting the risk of bias assessment.

The presentation of the quantitative summary – meta-analyses

Correlation between Delta 6 desaturase activity and the LA:DGLA ratio with serum or plasma Zn

The random-effect meta-analysis based on data from 7 studies (2 entries from Monteiro et al. 2021 study) demonstrated no significant correlation between delta 6 desaturase indices and Zn status (measured either as plasma or serum Zn, henceforth referred to as plasma zinc concentration, PZC) (Figure 2).

The pooled correlation coefficient was −0.0958, 95% CIs (-0.2912; 0.1074), $p = 0.2928$, and the heterogeneity was significant between the studies ($I2 = 81.7\%$, $p < 0.0001$). When the analysis was focused on studies that expressed delta 6 desaturase as the LA:DGLA ratio ($n = 5$), the pooled correlation coefficient tended to reach the significance threshold with a value of −0.1418, 95% CI (-0.2939; 0.0174), $p = 0.0686$, and the heterogeneity was not significant ($I2 = 49.3\%$, $p = 0.0957$) (Figure 3).

Association between the LA:DGLA ratio and Zn intake

The correlation estimates from 5 studies, revealed no significant pooled correlation coefficient between the LA:DGLA ratio and Zn intake with a value of −0.0215, 95% CIs (-0.1260; 0.0835), $p = 0.6007$.

The heterogeneity tests show the absence of heterogeneity among the studies (I^2=35.6%, $p = 0.1842$) (Figure 4).

The effect of Zn interventions on delta 5 desaturase activity

The random-effects meta-analysis indicated no significant mean difference between the intervention and control arm following a Zn intervention, with a pooled estimate value of 0.0609 95%CIs, (-0.1942; 0.3160), $p = 0.5437$ (Figure 5). The result of the heterogeneity tests tended to reach significance (I^2=52.8%, $p = 0.0759$).

The impact of Zn interventions on delta 6 desaturase activity

The pooled mean difference of delta 6 desaturase activity following a Zn intervention was not significant with a value of −0.0016, 95% CIs (-0.0066; 0.0035), $p = 0.4374$, and a noticeable heterogeneity between the studies (I^2=95.4%, $p < 0.0001$) (Figure 6).

When the analysis was based on studies that expressed the delta 6 desaturase activity with the same formula (GLA:LA, $n = 4$), there was no change in the pooled effect estimate, only in the confidence intervals: −0.0016, 95%CIs (-0.0091; 0.0058), $p = 0.5389$, with a high degree of heterogeneity (Figure 7).

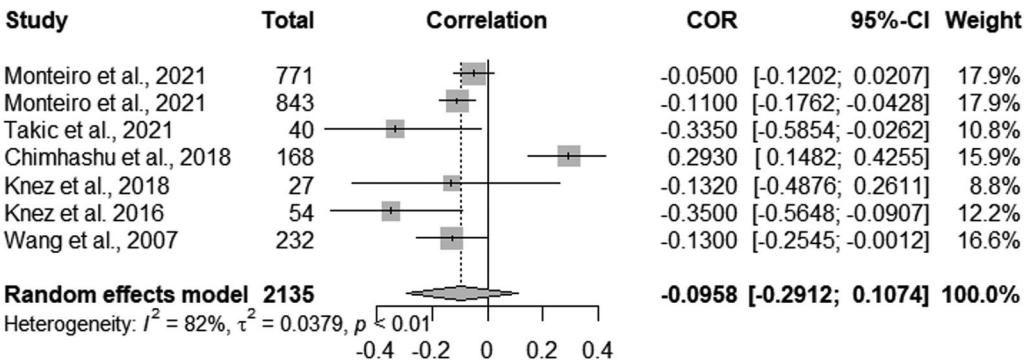

Figure 2. Meta analysis data.

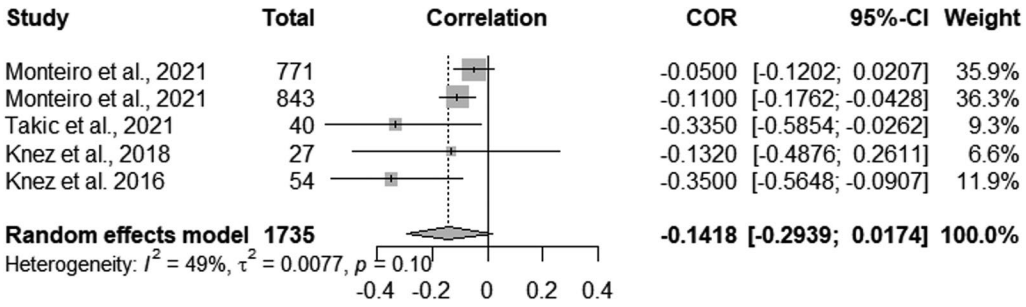

Figure 3. Meta analysis data.

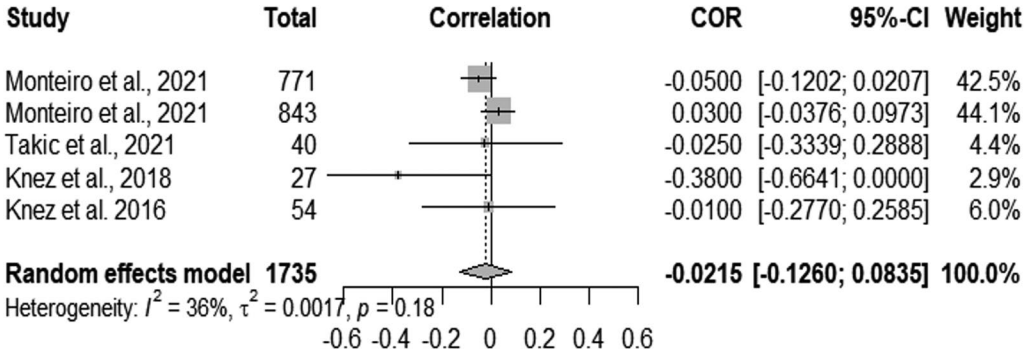

Figure 4. Meta analysis data

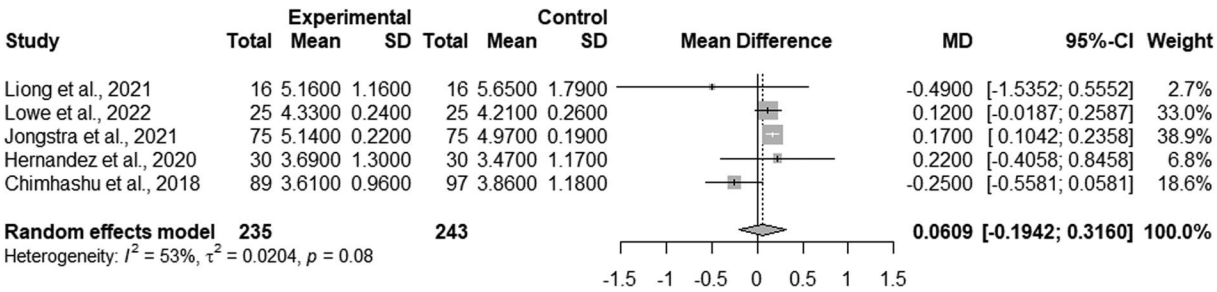

Figure 5. Meta analysis data

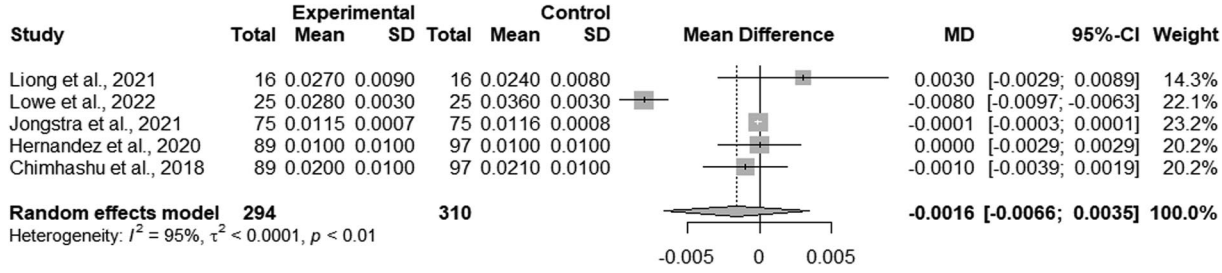

Figure 6. Meta analysis data

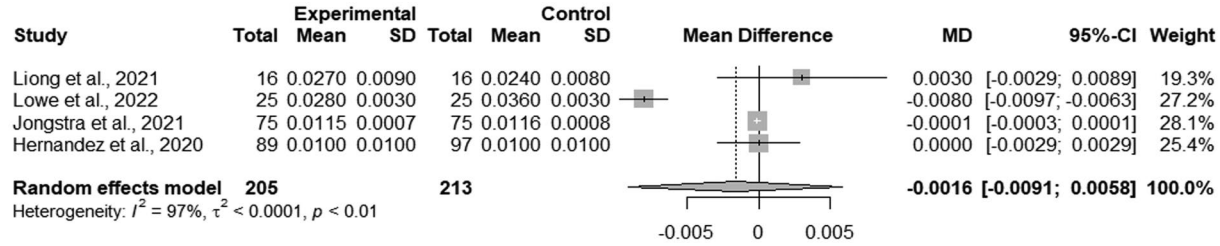

Figure 7. Meta analysis data

The response of delta 6 desaturase and Zn status to Zn interventions

For the purposes of this analysis, the standardized mean differences (SMDs) between the intervention and the control arm were pulled from studies that reported the change in delta 6 desaturase and plasma/serum Zn following a Zn-containing intervention. In both cases the pooled estimates were not significant; however, the value reflecting SMDs of delta 6 desaturase was higher: SMD for delta 6 desaturase was −0.6052, 95% CIs (−2.7162; 1.5058), p = 0.4289, while for plasma/serum Zn it was 0.0319, 95% CIs (−0.9133; 0.9770), p = 0.9213. In both cases the heterogeneity between the studies reached statistical significance (Figure 8a and b).

The LA:DGLA ratio and Zn status in relation to Zn intake

To distinguish which of the two biomarkers (LA:DGLA ratio or serum/plasma Zn) correlates more consistently with Zn intake, two separate meta-analyses based on identical study data were performed with pooled correlation coefficients between the LA:DGLA ratio and Zn intake and plasma Zn and Zn intake. The pooled correlation coefficient between the LA:DGLA ratio and Zn intake was non-significant with a value of −0.1050, 95% CIs (-0.5356; 0.3690), p = 0.454, and the tests indicated no significant heterogeneity among the studies (n = 3, I^2=28.3%, p = 0.2488). Similarly, the pooled correlation coefficient between plasma Zn and Zn intake was also statistically non-significant with a value of −0.0647, 95% CIs (-0.4224; 0.3106), p = 0.5453 and there was no heterogeneity among the studies (I^2=0.0%, p = 0.4174) (Figure 9).

The presentation of the qualitative synthesis – the descriptive analyses

Animal model studies

Two controlled studies completed on *Gallus gallus,* chicken model, used similar concentrations of Zn within a high Zn diet (46.5 µg Zn/g and 42.29 µg Zn/g, correspondingly). Knez et al. (2018) compared it to a 'low Zn' (32.8 µg Zn/g), while Reed et al. (2014) contrasted it to a 'Zn deficient' diet (2.55 µg Zn/g).

Both studies reported higher delta 6 desaturase activity (expressed as LA:DGLA ratio) in the group of birds on low or Zn deficient diets (Table 3). There was a statistically significant difference in the ratio between the two groups of animals at all time points, from day 7 to day 42 (Knez et al. 2018). The use of a Zn deficient diet contributed to a statistically significant increase in delta 6 desaturase activity on days 7, 14 and 21, but not on day 28 (Reed et al. 2014) (Supplementary Table 4).

Eder and Kirchgessner (1995) examined the effect using a rat model and various dietary Zn intakes on fatty acid desaturase activities in the presence of different types of dietary fats. There was no effect of any of the interventions on delta 5 desaturase activity, however, while there was no impact of Zn deficient diets on delta 6 desaturase activity in coconut oil-fed rats, the activity of this enzyme was higher in rats fed the flaxseed oil (Table 3). Interestingly, serum Zn levels were significantly lower in rats consuming a linseed oil compared to those fed coconut oil-based diet (Supplementary Table 4).

The effect of Zn particle size on fatty acid desaturase activities, was examined by Stawarska et al. (2021).

The same dose of Zn supplied as either micro- or nanoparticles was provided to rats and after 20 weeks, the activities of both enzymes were reduced in the group

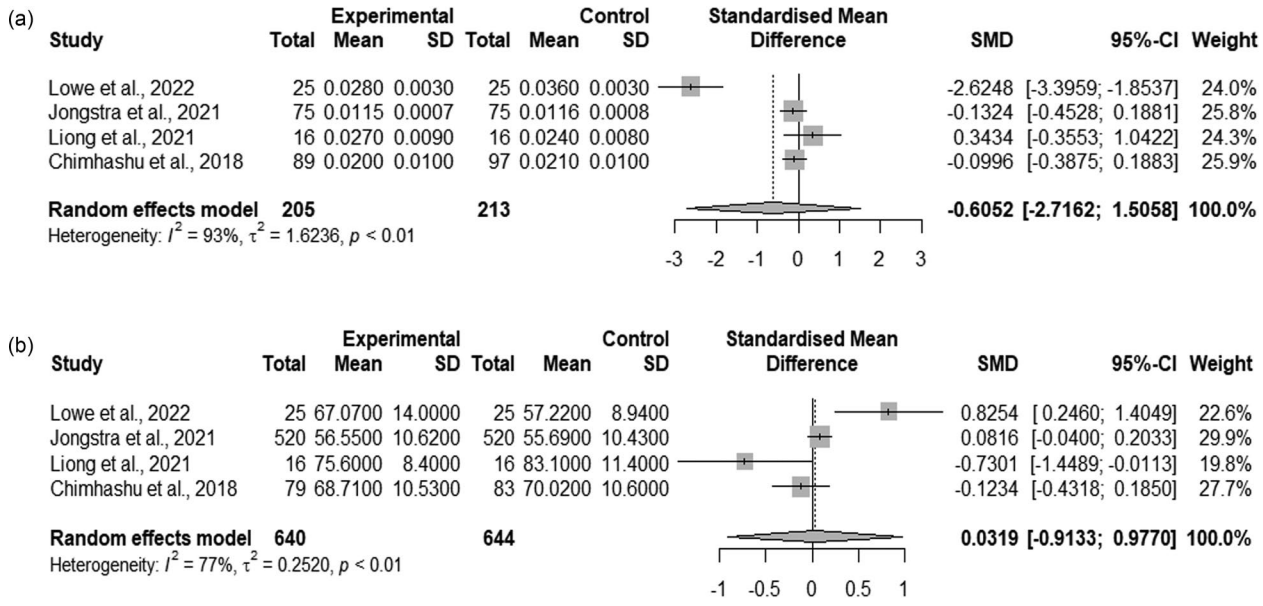

Figure 8. Meta analysis data.

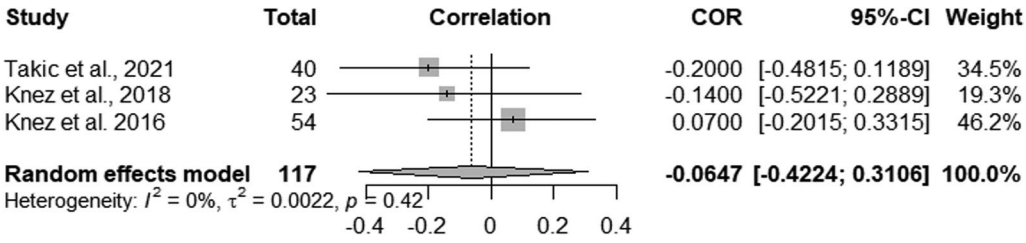

Figure 9. Meta analysis data.

receiving Zn microparticles, but the difference reached statistical significance only in the case of delta 6 desaturase.

Cunnane and Krieger (1988) applied high concentrations of Zn in the diet (up to 411 mg Zn/kg) and compared it to an optimal (36 mg Zn/kg) or a Zn deficient diet (3-4 mg Zn/kg). This was the only study that assessed delta 6 desaturase activity in specific subclasses of plasma and liver phospholipids and reported significantly higher enzyme activity in low Zn-fed rats compared to those receiving either optimal or high Zn diets.

Similar dietary Zn levels (50 ppm) were provided to animals by Ayala and Brenner (1983) over sixty days and by Kudo, Nakagawa, and Waku (1990) for two weeks. The Zn-deficient group of animals was switched to another diet after becoming severely ill, after 18 days (Ayala and Brenner 1983). Inconsistent findings were reported. Ayala and Brenner observed significantly higher values of both delta 5 and delta 6 desaturase in liver and testicular tissues in rats fed a Zn-adequate diet.

On the other hand, Kudo and coworkers reported significantly higher values of delta 6 desaturases isolated from liver phospholipids in rats fed Zn-deficient diets for 2 weeks. Clejan et al. (1982) who explored the difference in fatty acid desaturase activities in rats receiving a high Zn (100 ppm) or a Zn deficient diet (1.2 ppm) also observed an increase in the activity of both enzymes in liver microsomes in rats fed a high Zn diet for 6 weeks.

Human randomized controlled trials and non-randomized controlled trials

Out of five included RCTs (Table 1), three investigated the impact of Zn provided with food/water. Lowe et al. (2021) used Zn bio-fortified wheat as the intervention in a population of 50 women of reproductive age, Jongstra and coworkers (2021) examined the effects of Zn bio-fortified rice in 1-3-year-old Bangladeshi children, while Chimhashu et al. (2018) provided Zn fortified filtered water to school-age children.

None of the studies detected significant effects of employed Zn interventions on delta 5 and delta 6 desaturase activities after 8 weeks in women (Lowe et al. 2021), 9 months in toddlers (Jongstra et al. 2022), or 20 weeks in children (mean age of 8 years) (Chimhashu et al. 2018).

However, it was reported that plasma Zn status correlated significantly and negatively with the activity of FADS1 and positively with FADS2. Two other RCTs investigated the effect of the provision of Zn in the form of supplements – 25 mg of Zn as Zn gluconate provided to healthy men (Massih et al. 2021) and 30 mg of elemental Zn, as Zn sulfate, to T2DM patients (Hernandez et al. 2020). The first study had a different design as it investigated the impact of fasted/non-fasted state on desaturases activities, and it was shown that consumption of Zn supplements with breakfast led to a significantly higher activity of FADS1 with no

effect on FADS2 activity (Massih et al. 2021). On the other hand, the provision of Zn supplementation for 24 months did not induce any changes in desaturase activities in diabetic patients (Hernandez et al. 2020).

Finally, two depletion-repletion feeding trials investigated the effect of Zn biofortified wheat bread (Liong et al. 2021) and Zn biofortified rice (Suh et al. 2022) in healthy men. A decrease in FADS1 and an increase in FADS2 activities were seen after 6 weeks of consumption of Zn bio-fortified bread (6.3 mg/day) without additional phytate. In the final two weeks of the trial, participants received either 25 mg Zn/daily or a placebo along with the bio-fortified bread, but this did not alter the activities of the desaturases (Liong et al. 2021). The provision of 6 mg Zn/d (and 1.5 g phytate) for two weeks, and a 10 mg Zn/d diet for four weeks without phytate, provided in the form of cooked bio-fortified rice, significantly increased FADS1 activity in both cases. The FADS2 activity was higher only when Zn was provided over four weeks. The activities of both desaturases returned to the baseline value after three weeks of provision of an *ad libitum* diet supplemented with 25 mg Zn/d (Suh et al. 2022).

Human cross-sectional studies

The LA:DGLA ratio in relation to Zn intake/status

An association between the LA:DGLA ratio and Zn status/intake was examined in four studies (Table 2).

In a small-scale study in healthy subjects, the ratio had a significant negative correlation with Zn status and a non-significant negative relationship with Zn intake. while plasma Zn concentrations stayed unaffected, the LA:DGLA ratio responded to dietary Zn intake, the concentration of DGLA decreased, and the LA:DGLA ratio increased in people with lower dietary Zn intake (Knez et al. 2016).

Similar findings were provided by the NHANES study with more than 1500 participants, with a non-significant negative correlation between Zn intake and Zn status in both women and men (Monteiro et al. 2021). The other two studies were conducted on subjects with underlying chronic health conditions.

The one involving dyslipidemic subjects reported a tendency of a negative correlation between the LA:DGLA ratio and Zn status, while there was a statistically significant relationship between the LA:DGLA ratio and Zn intake (Knez et al. 2018).

Likewise, a study conducted on hemodialysis patients confirmed a negative association between the LA:DGLA ratio with Zn intake or Zn status (Takic et al. 2021).

The relationship between zinc status/intake and delta 5 desaturase activity

Three studies reported on the association between delta 5 desaturase activity and Zn status/intake and in all studies delta 5 desaturase activity was expressed by the same formula (AA/DGLA). In studies including patients with dyslipidemia and hemodialysis, the relationship between FADS1 and Zn intake was not significant, however, in the first study, the ratio reached a positive (Knez et al. 2018), while in the other it had a negative direction (Takic et al. 2021). A non-significant negative correlation was observed between dietary Zn intake and enzyme activity (Takic et al. 2021). In a Finnish population study that included diabetes-free men no correlation analyses were performed, however, the authors reported that delta 5 desaturase activity was the highest in the first Zn status quartile, and lowest in the last quartile (Yary et al. 2016).

The association between zinc status/intake and delta 6 desaturase activity

The correlations between delta 6 desaturase activity and Zn status/intake were assessed in four studies (Wang et al. 2008; Yary et al. 2016; Knez et al. 2018; Takic et al. 2021), with desaturase activities expressed by different formulas (GLA:LA or AA:LA or GLA:AA). Among 40 patients undergoing hemodialysis, desaturase activity was directly correlated with Zn status, while there was no relationship with dietary Zn intake (Takic et al. 2021). Also, there was no association between delta 6 desaturase and Zn status in dyslipidemic subjects (Knez et al. 2018). On the contrary, Wang et al. (2008) found a non-significant but negative correlation between FADS2 and Zn status.

Finally, Yary et al. (2016) reported that the lowest delta 6 desaturase activity was observed in the first while the highest was measured in the last quantile of Zn status in 2189 men from the prospective Kuopio Ischemic Heart Disease Risk Factor Study in Eastern Finland.

Influence of Zn on elongase and nervonic acid

The effect of Zn on elongase and nervonic acid were reported in 4 RCTs and 1 cross-sectional study (Supplementary Table 5). Lowe et al. (2021) reported in their cross-sectional RCT in women of reproductive age that 8 weeks of intervention with Zn bio-fortified wheat induced a significant decrease in elongase activity (expressed as DGLA:LA) in one of the arms, not in both. Furthermore, the study involving 520 toddlers reported no significant effects of consumption of Zn bio-fortified rice for nine months on either elongase or nervonic acid activity (Jongstra et al. 2022). In Zn deficient African children Zn fortification significantly increased nervonic acid content(Chimhashu et al. 2018).

However, no effect on elongase activity (expressed as AA/LA) was seen. In the cross-sectional study involving patients undergoing hemodialysis, elongase activity showed a non-significant direct correlation with both Zn status and Zn intake (Takic et al. 2021). The higher elongase activity was measured in subjects receiving Zn supplementation before breakfast, compared to those who received it along with breakfast (Massih et al. 2021).

Discussion and suggestions for further research

This systematic review aimed to provide an in-depth investigation of the relationship between fatty acid desaturases

and Zn (measured as intake/status) based on the available research evidence. The qualitative synthesis was also supported with the quantitative analysis where deemed appropriate. Overall, the meta-analyses did not reveal any significant pooled estimates between Zn status/intake and desaturases. However, some speculations can be derived based on these analyses: LA:DGLA ratio correlates more strongly with plasma Zn status and Zn intake compared with desaturase 6 when the formula is expressed with other fatty acids; desaturase 6 appears to provide a greater response to Zn-containing intervention than Zn status. The descriptive analysis on the other hand, gave important information related to factors that potentially mediate the response of desaturases to Zn interventions. Animal study data yielded insights into the importance of different confounders including: the type of dietary fat, the size of Zn particles, the quantity and the type of food provided (ad libitum vs restricted diet). Cross-sectional studies reported inconsistent results regarding the correlations between Zn intake/status and desaturases, however, the negative coefficient was observed more often. In addition, gender seems to influence these results, as opposite trends of correlations were reported in men and women, while underlying chronic conditions appear not to influence the relationship between dietary Zn intake and LA:DGLA ratio. Finally, the findings from the RCTs and feeding trials are not consistent as well however this might be explained by the difference between the study populations, durations, the type of dietary interventions. One of the important confounders derived from these studies is the way in which Zn supplements are provided (fasted state vs with food).

Several important conclusions can be drawn from the meta-analyses. No significant correlation was seen between the delta 6 desaturase and Zn status. Though the meta-analysis did not reveal a significant correlation coefficient between the LA:DGLA ratio and Zn intake/status, the magnitude of the pooled estimate considerably increased when the analysis included only studies that expressed delta 6 desaturase activity as the LA:DGLA ratio, and in this case the relationship with Zn intake tended to reach statistical significance. This result suggests indirectly that the LA:DGLA ratio correlates more strongly with plasma Zn status and Zn intake than the ratio of other examined fatty acids.

The pooled estimates of desaturase activities were not significantly affected by different Zn-containing interventions, and this was not changed when the analysis focused only on studies that used the same formula of delta 6 desaturase, suggesting that in this case the formula for estimating desaturase 6 was not of importance. When we aimed to explore quantitatively whether delta 6 desaturase responds in a greater magnitude than Zn status to Zn-containing interventions by performing 2 separate meta-analyses on the same studies, we observed that in none of the cases there was a significant pooled SMD, however, the magnitude of SMD was greater in the case of delta 6 desaturase. We might speculate that the response of delta 6 desaturase to Zn interventions is greater than the response of Zn status however this observation should be further explored further.

Finally, two separate meta-analyses on same studies that assessed the correlations between LA:DGLA and Zn intake and Zn status and Zn intake revealed that in both cases the direction of correlations was negative and that the magnitude was only slightly different. Thus, we could contemplate that the LA:DGLA ratio is a more reliable parameter that more consistently correlates with Zn intake compared to Zn status.

All meta-analyses results should be interpreted with caution. Studies for which raw data was not available had to be excluded from the analysis. Results of non-significant tests should be taken with caution as it is known that a small number of studies can introduce bias in these tests (von Hippel 2015). In addition, diversity in study designs, population groups, the health status of participants, ethnicity, age, gender, study duration, and variety of Zn interventions explain the heterogeneity among studies.

The fact that a few different factors such as type of fat, supplementation in fasted or fed state, the size of the Zn particles, influence the activities of desaturases, reinforces the statement that the results obtained by meta-analyses should be interpreted with additional care, as the available data varied in the type of employed Zn interventions, in the first place.

It should also be noted that the heterogeneity disappeared when the analysis included only studies reporting the correlations between the LA:DGLA ratio and Zn intake/status. In contrast, the heterogeneity increased when additional studies that expressed delta-6 desaturase activity with different formulas were included in the analysis, indicating that the expression formulas used for desaturase determination might be important factors of variability between the studies.

Therefore, establishing straightforward recommendations for assessing desaturase activities in Zn intervention trials might help in providing more homogenous data that will strengthen the evidence on the diagnostic performance of FADS1 and FADS2 in relation to Zn intake.The quality assessment also yielded some valuable insights as certain RCTs were assessed as having a 'high' risk of bias, simply because the information of interest on the methodology part of the manuscript was not provided. Thus, future efforts in reporting on RCTs should involve all necessary elements and aim to adhere to the recommended guidelines for publishing RCTs. This was the case with animal studies as well. The analysis of animal study data has only started to gain attention and practice during the last two decades, since an influential Lancet commentary pointed to the scientific rationale of doing systematic reviews based on data taken from animal model studies (Sandercock and Roberts 2002).

The existing evidence strongly points out that many publications based on animal model studies do not contain all relevant methodological information, thus, animal studies require improvements in terms of describing their methods and results comprehensively and transparently (Kilkenny et al. 2009; Macleod et al. 2015). The ARRIVE guidelines could be used as a guiding tool by both authors and journals to ensure that all relevant points suggested by the reporting

checklist tool for reporting in vivo experiments are covered (Kilkenny et al. 2010).

The results from the descriptive analysis also provided valuable insights regarding the relationship between Zn and FADS1 and FADS2 activity. The link between dietary Zn intake and FADS1 and FADS2 activity was initially explored using animal model studies, both Sprague-Dawley and Wistar rats in the early 1980s (Clejan et al. 1982; Ayala and Brenner 1983;Cunnane and Krieger 1988). Consistent findings were provided; in general, the activity of both enzymes was reduced as a consequence of Zn deficiency (Clejan et al. 1982; Ayala and Brenner 1983; Cunnane and Krieger 1988; Kudo, Nakagawa, and Waku 1990). Delta 6 desaturase activity did not differ between Zn-deficient and Zn-adequate rats fed coconut oil but it was higher in Zn-deficient rats fed linseed oil diet, pointing out that the type of dietary fat has a role to play in the process (Eder and Kirchgessner 1995). Diets with high levels of polyunsaturated and saturated fatty acids inhibit, while fat-free diets stimulate the activity of desaturases (Eder and Kirchgessner 1995).

Similar findings were reported by others, high-fat diets inhibited activities of liver delta 5 and delta 6 desaturases in rats (Valenzuela et al. 2015).

This was confirmed in humans, provision of high fat diet for several weeks suppressed delta 6 desaturase activity in postmenopausal women (Raatz et al. 2012). Hence, proper dietary data collection and qualitative assessment of dietary fat intake are quite important in this instance, as it should ensure that observed changes in desaturase activity are entirely caused by dietary Zn modifications, rather than differences in other dietary components.

Furthermore, tissue-specific regulation of desaturases seems to exist, as confirmed by different expressions of desaturases in adipose and liver tissues of animals (Eder and Kirchgessner 1995).

Further research is necessary to differentiate FADS1 and FADS2 activity in hepatic from the expression and activity of desaturases in non-hepatic tissues.

Another important factor reported to affect the response of desaturases to Zn interventions is the size of Zn particles. Delta 6 desaturase was significantly lower in subjects receiving Zn in microparticles compared to the group in receipt of Zn in nanoparticles (Stawarska et al. 2021). The quantity and the type of food provided are relevant; rats receiving a diet with adequate Zn content, but in limited amounts expressed the highest level of delta 6 desaturase activity (Clejan et al. 1982; Eder and Kirchgessner 1995).

Similar results were obtained when chicken, *Gallus gallus*, was used as a model, the erythrocyte LA:DGLA ratio distinguished Zn status between Zn-adequate and Zn-deficient subjects and a significant negative correlation was found between dietary Zn deficiency and the LA:DGLA ratio (Reed et al. 2014; Knez et al. 2018). Additionally, changes in the LA:DGLA ratio were apparent after seven days only, thus demonstrating that the ratio can show variations in Zn status rapidly and has the potential to reveal early stages of Zn deficiency (Reed et al. 2014; Knez et al. 2018).

The use of different formulas for expression of desaturase activities, employment of various methods of their estimation, dissimilar doses of Zn and diverse duration of studies, and the fact that Zn was measured in various tissues (liver, hepatic microsomes, serum, testes tissues, erythrocytes) made data interpretation, comparison, and analysis of findings reported by animal trials challenging.

Although it was not possible to perform a meta-analysis of these studies, the results demonstrate that desaturases respond to dietary Zn modifications and that FADS1 and FADS2 activity has the potential to act as an additional biomarker of Zn status in animals.

In cross-sectional studies, statistically significant differences were not always seen, but a negative trend was always present in correlations between dietary Zn intake and activity of certain desaturases (Knez et al. 2016; Yary et al. 2016; Monteiro et al. 2021). Negative correlations between FADS2 activity with Zn intake and Zn status and both positive and negative correlations for FADS1 activity were reported. FADS2 activity was lowest and FADS1 activity was highest in the first Zn status quartile (Yary et al. 2016). Negative associations between the LA:DGLA ratio and Zn status were seen in women, while a positive interaction was found in men within a single study (Monteiro et al. 2021), which suggests that gender may be a confounding factor in the relation. As demonstrated by others, a higher contribution of the n-6 essential fatty acids to plasma total lipids and plasma phospholipids was shown in women than in men (Lohner et al. 2013). Similarly, women have a higher capacity for fatty acid synthesis (Extier et al. 2010). Estrogen promotes, while testosterone decreases the transport of essential fatty acids into their longer-chain metabolites (Childs et al. 2010). Thus, gender differences should be taken into consideration when the diagnostic performance of desaturases to Zn intake is examined.

Furthermore, certain ethnic differences in desaturase activities were reported, delta 6 desaturase activity was found to be significantly lower in Asian Indian women compared to white Europeans (Gray et al. 2013). Delta 5 activity was higher in the African Caribbean compared with white European women (Merino et al. 2011). A comparison of reported delta 6 desaturase levels among studies in White Caucasians (Knez et al. 2018) and Asians (Wang et al. 2008) demonstrate lower levels of delta 6 desaturases in Asians.

Besides, a direct negative trend between the LA:DGLA ratio and dietary Zn intake was also reported for subjects with underlying chronic health conditions (Wang et al. 2008; Yary et al. 2016; Knez et al. 2018; Takic et al. 2021), meaning that the biomarker is less likely to be affected by infectious and inflammatory conditions.

Lower levels of desaturase activity are measured in people with an elevated risk of cardiovascular diseases, T2DM, obesity, hypertension, hyperlipidemia, and chronic inflammatory disease (Fujita et al. 2012; Mayneris-Perxachs et al. 2014; Zhao et al. 2016; Svendsen et al. 2020). However, it is uncertain if dysregulation of desaturases, frequently linked to decreased Zn levels, is a cause or a consequence of metabolic disturbances, which requires further investigation.

Further well designed, methodologically sound, human randomized controlled Zn intervention trials are needed to explain the impact infection and inflammatory conditions might have on FADS1 and FADS2 diagnostic performance.

However, inconsistent findings were provided by RCTs. No significant effect of Zn treatment on delta 6 desaturase activity was seen in children fed Zn biofortified rice (Jongstra et al. 2022), while a decrease in delta 6 activity was evident after six to eight weeks of provision of high Zn biofortified wheat diet to both women and men (Liong et al. 2021; Lowe et al. 2021). Some feasible factors can be offered to help explain the observed differences. For instance, a much lower dose of Zn was provided by meals based on Zn biofortified rice vs. wheat relative to the corresponding controls. Similarly, there might have been differences in the relations between Zn intake and FADS1-2 activities because of age related differences (children vs. adults). In this regard, reduced delta-6 desaturase activities as a result of aging have been shown (Cho, Nakamura, and Clarke 1999; Liu and Medeiros 1995) and age should therefore be included as a potential covariate. It is important to mention that desaturases responded to dietary Zn modifications while there were no measurable effects in plasma Zn levels, glutathione concentrations or DNA strand breaks (Liong et al. 2021) confirming that desaturase enzymes are more sensitive to slight changes in dietary Zn intake compared to other biomarkers.

Furthermore, Zn status plays a key role in FA desaturation and/or elongation and the LA:DGLA ratio can be used as a biomarker of Zn status (Chimhashu et al. 2018). Zn supplementation affected fatty acid desaturation only when supplemental Zn was provided with food and increased plasma Zn concentrations were seen only when Zn supplements were taken without food (Massih et al. 2021).

This finding clearly demonstrates that the way Zn is provided, in a fasted state or with food, is an important factor (Massih et al. 2021). Zn taken without the food is transported to peripheral blood, whereas Zn absorbed with food turns out in the liver via the portal circulation (Lowe, Woodhouse, and King 1998). The form and amount of Zn and the way it is provided, with or without food, should be accurately specified in Zn intervention trials before desaturase activity is investigated.

There was no effect of Zn bio-fortified rice on elongase activity or nervonic acid levels in children except in the Zn deficient group of kids provided with Zn fortified water (Chimhashu et al. 2018). Elongase activity tended to be higher in subjects receiving Zn supplementation before breakfast vs. those who received it with breakfast (Massih et al. 2021).Zn/Cu ratio was directly associated with elongase activities in hemodialysis patients (Takic et al. 2021). Increased concentrations of Cu are correlated with improved delta 6 desaturase activity (Ho, Elliot, and Jones 1975; Knez et al. 2018). Cu/Zn ratio is frequently altered in patients with metabolic disturbances and in these cases, Cu often takes over Zn's position, thus investigation of the relations between Zn intake and fatty acid metabolism in unhealthy cohorts should include an analysis of both nutrients.

Similarly, it appears that a minimum of six weeks is needed to observe the changes in desaturase activity in response to Zn interventions, however, this requires to be confirmed with additional, for this purpose, properly designed RCTs. Further studies should examine the effectiveness of desaturases to, precisely and accurately, describe changes in bioavailable Zn intake over time, i.e., low vs. high Zn intake and over long vs. short provision times.

This systematic review has several strengths. It included a wide variety of studies with different designs, studies with a relatively large number of human subjects and studies performed in various geographical locations; therefore, the obtained results may be more widely applicable.

In addition, the analyzed studies were carried out over three decades, using different human and animal experimental models, indirectly supporting the contention that the original idea of the role of Zn in fatty acid metabolism has been reinforced over time. The meticulous analysis allowed identification of some potential covariates and confounders of observed relations and recognition of limitations and weaknesses in reporting both animal and clinical trials, which could be addressed in future research efforts.

There are also some weaknesses, the majority of available studies were observational and not randomized controlled trials, so the quality control potential of the studies included in the review is limited to an extent. There were not many randomized controlled trials, which represent the golden standard among study designs. The analytical methods for fatty acid analysis were not standardized among the research groups, a few different calculations were used to assess the activities of certain desaturases, which made the process of evaluating effects and interpretating findings challenging. In addition, the results of the meta-analysis should be interpreted with caution, due to the heterogeneity among the analyzed studies. However, our main aim was to summarize all relevant animal and human studies that explored the response of fatty acid desaturases to Zn, and despite the study heterogeneity, we believe that, along with the summarized results, the limitations derived from these studies also offer valuable implications for research groups when designing future studies aiming to address this question.

Lastly, a number of factors may influence fatty acid metabolism, and Zn intake relations i.e., dietary intake of fats, gender, age, ethnicity, health status and Zn particle size. Based on the currently available data, the prospective contribution of some identified confounders and covariates on observed relations could not be determined, so further research in this area is needed. Furthermore, the diagnostic performance of FADS1 and FADS2 in relation to Zn intake should be examined in population groups with and without Zn deficiency, healthy and unhealthy cohorts, adjusting for all associated covariates and confounders as much as possible. Finally, an appropriate randomization process and precisely defined study entry criteria are required to exclude both recognized and unrecognized covariates and confounders.

Conclusions

This systematic review provides a complete comprehensive summary of major findings, and some novel insights, on FADS1 and FADS2 activities in relation to Zn intake. More specifically, the meta-analysis revealed a non- significant correlation between the delta 6 desaturase and Zn status. On the other hand, delta 6 desaturase seems to respond in a greater magnitude than Zn status to Zn-containing interventions as suggested by its' higher value of SMD. Finally, two separate meta-analyses with the same studies used to assess the correlations between LA:DGLA and Zn intake and Zn status and Zn intake revealed that these two biomarkers correlated similarly with Zn intake. The findings obtained from the meta-analysis provide a more reliable insight into the activity of fatty acid desaturases and their relationship with Zn when compared with single studies, however, it should be emphasized that a considerable heterogeneity between the studies was present. Thus, further experimental animal model studies and clinical trials are necessary to additionally evaluate the observed interrelations and to validate the efficacy of desaturase enzymes to respond to changes in dietary Zn intakes. Future studies should also take into account the findings derived from the qualitative synthesis concerning the confounders that mediate the activity of fatty acid desaturases in response to Zn-containing interventions.

The work provided should be used as a guideline for conducting future clinical trials that will explain the proposed relationships further and assist in confirming the efficacy of the FADS1 and FADS2 activity to act as Zn status indicators, individually or in combination with some other biochemical markers.

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