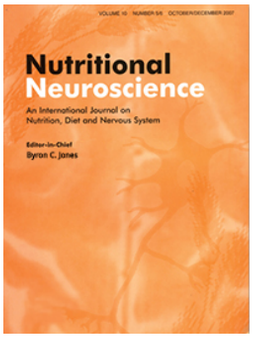

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REVIEW

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The effect of vitamin D supplementation in treatment of children with autism spectrum disorder: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Objective: The effect of vitamin D supplementation on the risk of Autism Spectrum Disorder (ASD) is conflicting. The aim of this study was to estimate the efficacy of vitamin D supplementation on ASD in children.

Methods: We conducted a meta-analysis of randomized controlled trials (RCTs) in which vitamin D supplementation was used as a therapy in children with ASD. The PubMed, PsychINFO, Cochrane CENTRAL library, Web of Science, and Cinahl databases were searched from inception to March 20, 2019, for all publications on vitamin D and ASD with no restrictions. Studies involving individuals aged <18 years diagnosed with ASD and with all functional outcomes assessed by measurement scales for ASD were included. Mean differences were pooled, and a meta-analysis was performed using a random-effects model due to differences between the individual RCTs.

Results: There were five RCTs with 349 children with ASD in the review, of which three RCTs were included in the meta-analysis. Vitamin D supplementation indicated a small but significant improvement in hyperactivity scores (pooled MD: −3.20; 95% CI: [−6.06, −0.34]) with low heterogeneity ($I^2 = 10\%$, $p = 0.33$), but there were no other statistically significant differences in ASD symptoms between groups as measured by validated scales.

Conclusion: Vitamin D supplementation appears to be beneficial for hyperactivity but not for core symptoms or other co-existing behaviors and conditions of ASD. Future RCTs with large sample sizes examining the effect of vitamin D supplementation on ASD among individuals with low serum vitamin D levels at baseline are needed.

KEYWORDS

Vitamin D supplementation; autism spectrum disorders; children; randomized controlled trials; meta-analysis; systematic review; hyperactivity; docosahexaenoic acid; core symptoms

Introduction

Autism spectrum disorder (ASD) is a range of early-onset neurodevelopmental disorders characterized by difficulties with social interaction, impairments in verbal and non-verbal communications, and stereotyped repetitive behaviors[1]. The global prevalence of ASD has dramatically increased over the past few years and is currently about 1 in 59 children[2]. The rising ASD rates are ascribed to complex interactions between multiple genes and environmental components[3,4]. Recent epidemiological studies indicate that concentrations of vitamin D are decreased in children with ASD compared to typically developing children[5–7]. Vitamin D acts as a neuroactive steroid and plays an important role in brain development and mature brain function, and vitamin D receptors have also been found in the human brain[8,9]. Pre-clinical work in animal models also indicates that vitamin D deficiency induces several changes in brain systems[10,11]. These epidemiological and experimental data have prompted several randomized controlled trials (RCTs) to determine whether vitamin D supplementation can decrease the risk of ASD. However, the results of the RCTs are inconsistent. For example, Mazahery et al.[12] showed that supplementation with vitamin D was superior over placebo in reducing irritability and hyperactivity[12], while Kerley et al.[13] did not find an effect of vitamin D on the severity of ASD symptoms[13]. Recently, Song et al.[14] examined the efficacy of vitamin D supplementation in children with ASD through a meta-analysis[14]. However, it did not

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specially focus on symptoms of ASD such as social interaction, communication, and repetitive restrictive behaviors or interests (RRB) or symptoms or behaviors associated with ASD, including hyperactivity, irritability, and sensory issues. In addition, the Cochrane handbook for systematic reviews of interventions pointed out that comparison of changes from baseline will enhance the statistical power and be more efficient than comparison of post-intervention values, which were analyzed in Song's study[15].

Given the discrepancies in the included studies and the public health importance of clarifying the role of vitamin D in ASD, a meta-analysis on this subject is warranted. The purpose of this study was to perform a meta-analysis of RCTs measuring the effectiveness of vitamin D supplementation in children with ASD compared with healthy controls.

Methods

Search strategy

This systematic review and meta-analysis of the literature was performed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[16]. To ensure a comprehensive search of the literature, keywords and their combinations were searched in multiple electronic databases including PubMed, PsychINFO, Cochrane CENTRAL library, Web of Science, and Cinahl from their date of inception to March 20, 2019, with no restrictions.

The search terms were as follows:'(vitamin D OR cholecalciferol OR ergocalciferol) AND ((vitamin D) AND (supplements OR supplementation OR intake)) AND (autistic disorder OR autism OR autism spectrum disorder OR Asperger)'. Reference lists of the original studies and reviews were scanned for additional studies. The search was limited to English articles, but no other restrictions were imposed.

Inclusion and exclusion criteria

Two investigators independently scrutinized the electronic searches and obtained the full articles for all studies that were potentially eligible for inclusion. Full-length articles of studies evaluating the effectiveness of vitamin D supplementation in children diagnosed with ASD were examined and subsequently selected if they fulfilled the following inclusion criteria: (1) the design was an RCT, (2) the population was children aged <18 years whose primary diagnosis was ASD based on established criteria, (3) the protocols were specified for vitamin D supplementation only or vitamin D combined with other vitamins in children with ASD and placebo or no supplementation in the control group, and (4) there was at least one outcome measure, including core symptoms of ASD such as social interaction, communication, and RRB or symptoms or behaviors associated with ASD, including hyperactivity, irritability, and sensory issues.

Studies were excluded if they were individual case studies, conference abstracts, observational studies, proceedings without a related publication, reviews or meta-analyses of the literature, duplicate reports, assessments of autistic traits/behaviors rather than ASD diagnosis, or did not provide necessary data. Disagreements were resolved through discussion until consensus was reached.

Quality assessment

The methodological quality of each eligible RCT was evaluated using the Cochrane Risk Assessment Tool by one researcher and checked by another[15]. The following seven items were assessed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. Each item was assessed as having low, high, or unclear risk of bias, and all studies were assigned a summary score for study quality as assessed across studies. Disagreements were resolved through discussion until consensus was reached.

Data extraction and synthesis

The following information was independently extracted from the study reports by two researchers: the first author's last name, year of publication, country of origin, total sample size, sex distribution, age of children when the outcomes were assessed, vitamin D supplementation dose and duration, serum 25-hydroxyvitamin D (25 (OH)D) level at baseline, the diagnostic criteria that were used, and information about study quality.

Statistical analysis

For each outcome, the mean change and standard deviation (SD) of change from baseline to endpoint for each intervention group (vitamin D and placebo) were entered into RevMan version 5.3 (Cochrane Collaboration) for analysis. If only baseline and end data were available, the mean change was calculated by subtracting the baseline value from the end value, and the SD was then imputed from the mean correlation coefficient for an outcome from other studies in the meta-analysis.

The primary meta-analysis was performed using random effects due to differences between the individual RCTs. Mean differences (MDs) with 95% CIs (net changes in scores) were calculated for continuous outcomes (ASD symptom scale) between the vitamin D and control groups. Heterogeneity between included studies was assessed using both the Chi^2 statistic ($p < 0.1$ showed substantial or considerable heterogeneity) and the I^2 statistic (I^2 0%–40%, low; 30%–60%, moderate; 50%–90%, substantial; 75%–100%, considerable heterogeneity)[15]. No subgroup analyses or meta-regressions were performed due to the limited number of studies included in the meta-analysis. In addition, publication bias was not assessed because very few studies contributed data.

Results

Search results and study selection

A total of 425 articles were found in our initial search, and 89 were duplicates. After removal of duplicates, 317 were excluded by screening abstracts or titles leaving 19 articles to be assessed for eligibility. After reading the full text, five studies met the inclusion criteria and three were included in the final analysis[12,13,17]. Two studies were included in the overall interpretation but not included in the meta-analysis due to the use of an assessment tool not used by the others[18,19]. The reasons for excluding the 14 full texts were as follows: 8 did not answer our systematic review question[20–27], 1 was retracted by the journal due to incorrect data[28], 2 were case reports[29,30] and 3 were open-label trials[31–33]. The screening and selection of the studies are presented in the PRISMA flowchart shown in Figure 1.

Study characteristics

Table 1 summarizes the characteristics of the included studies. Among them, two studies were conducted in New Zealand[12,17], one in Egypt[18], one in Iran[19] and one in Ireland[13]. All five studies were published in English between 2014 and 2019, and the five selected trials recruited 349 participants aged 2–12 years diagnosed with ASD (309 were male and 40 were female). Vitamin D supplementation was in the form of cholecalciferol in all five RCTs. For the intervention group, the daily doses were 2,000 IU/day in four RCTs, and the remaining study used 300 IU/kg/day not to exceed 5,000 IU/day[19]. All included studies except one were placebo-controlled. All studies included both males and females, except for the Moradi, 2018, study[19] that only included males. The male to female ratio ranged from 3/1–7/1. The interventions lasted 3 months (one RCT), 5 months (one RCT), 6 months (one RCT), and 12 months (two RCTs).

The Aberrant Behavior Checklist (ABC) was the scale most commonly used to evaluate the behaviors of children, but the Childhood Autism Rating Scale (CARS), the Autism Treatment Evaluation Checklist (ATEC), the Social Responsiveness Scale (SRS), the Sensory Processing Measures (SPM), the Developmental Disabilities-Children's Global Assessment Scale (DD-CGAS), and the stereotypy subscale of the Gilliam Autism Rating Scale-Second Edition (SSGARS-2) were also used. Of the five RCTs included, full data were available for only three RCTs using the same assessment tool (ABC or SRS) and only these three were included in the final meta-analysis [12,13, 17].

Risk for bias in the included studies

Figure 2 shows the risk for bias. Two studies[18,19] that did not mention the method of random-sequence generation were considered to have unclear risk for randomization bias. Other studies used computer generated numbers and block randomization stratified by age and severity of ASD[12,13,17]. Three studies[13,18,19] were assessed as having unclear risk of allocation concealment bias owing to insufficient information. The other two trials used a third party for randomization[12,17]. Only two studies were reported as double-blinded (researchers and children/caregivers)[12,17]. One study was reported to have high risk of performance bias[18]. It is unclear if researchers/assessors and participants were blinded in the other two studies[13,19]. The blinding was maintained throughout the trial until after data analysis in only two trials[12,17], and no details were provided for the blinding of outcome assessment in the other three studies[13,18,19]. There were low rates of loss to follow-up in all five studies. All outcomes in all trials were reported. The potential for other biases was not apparent in any study.

Effect of vitamin D on core symptoms of ASD

Social interaction: Of the two studies testing the effect of vitamin D supplementation on ASD (assessed using the ABC), one study examined both vitamin D alone and vitamin D together with docosahexaenoic acid (DHA) supplementation[12]. In this case, the study could be considered as two independent reports. Thus, there were three independent RCTs involving 104 participants included in this meta-analysis. The pooled effect estimates for social interaction did not differ between the intervention and control groups in changes of mean scores (pooled MD: −1.54; 95% CI: [−4.09, 1.01]; $p =$

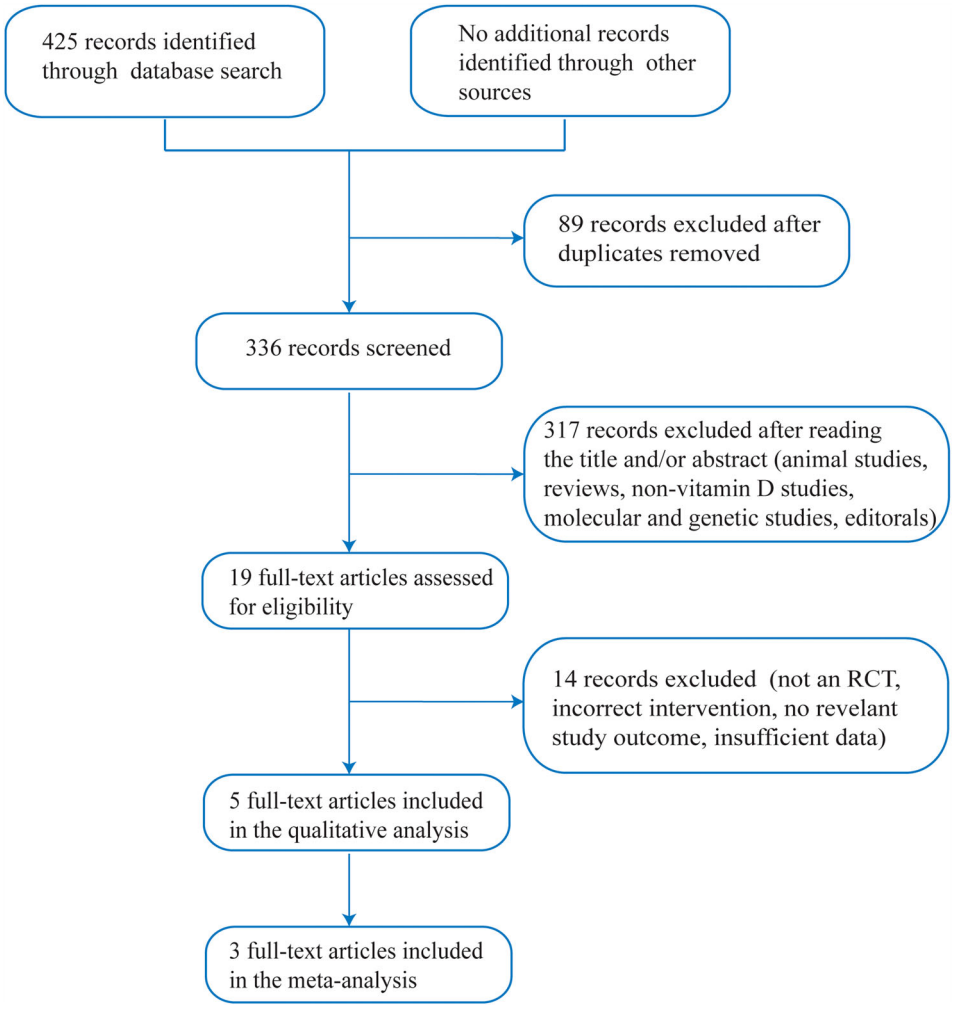

Figure 1. Flow diagram for selection of studies (PRISMA flow diagram).

0.24), and no heterogeneity was seen (Figure 3(a)). The mean change scores decreased (showing an improvement) in all intervention groups in these studies. Removing the study[12] that examined vitamin D together with DHA supplementation did not change the results. Additionally, one RCT[18] that was not included in the meta-analysis did not find any effect of vitamin D supplementation on social interaction (as measured by the CARS and ATEC, all $p > 0.05$).

Using the SRS social interaction sub-domains, the Mazahery, 2019, study[17], which tested the effect of vitamin D alone and together with DHA supplementation, found no effect of vitamin D supplementation on social interaction such as social skills (pooled MD: −5.80; 95% CI: [−13.67, 2.07]; $p = 0.15$), social awareness (pooled MD: −1.26; 95% CI: [−2.77, 0.25]; $p = 0.10$), social cognition (pooled MD: 0.31; 95% CI: [−1.44, 2.05]; $p = 0.73$), or social motivation (pooled MD: −0.90; 95% CI: [−3.02, 1.21]; $p = 0.40$) and found no heterogeneity (Figure 4(a)).

Similarly, Kerley *et al.*[13] and Mazahery *et al.*[17] also used other tools, including the DD-CGAS and the social interaction sub-domain of the SPM, respectively. Neither study found any effect of vitamin D supplementation on social interaction.

Communication: There were no significant differences between groups in changes of mean scores for communication as assessed using the ABC (pooled MD: −0.05; 95% CI: [−1.79, 1.69]; $p = 0.96$), and moderate heterogeneity was found in the meta-analysis ($I^2 = 60\%$, $p = 0.08$) (Figure 3(b)). Removing the study[12] that tested the effect of vitamin D/DHA together had no impact on the overall result.

Mazahery et al.[17] also used the communication sub-domain of the SRS to assess of the effect of vitamin D supplementation alone or combined with DHA on communication. There were no significant differences between groups in changes of mean scores (pooled MD: −2.53; 95% CI: [−6.66, 1.61]; $p = 0.23$), and no heterogeneity was seen in the meta-analysis ($I^2 = 0\%$, $p = 0.87$) (Figure 4(b)). Likewise, Kerley *et al.*[13] did not find any effect of vitamin D supplementation on communication using the DD-CGAS tools.

Table 1. Characteristics of the RCTs included in the systematic review.

RCTs included in the meta-analysis ($n = 3$)								
Author, year	Country	Total	Sex distribution (M, F)	Age (years)	Baseline serum 25(OH)D (nmol/L)	Treatment	Dose	Duration	Outcome measure	Study quality
Mazahery, 2018[12]a	New Zealand	73	60M, 13F	2.5–8 (5.2, mean)	Mean: 68 ± 21	Vitamin D3	2000 IU/day	12 months	ABC	RDBPC
Mazahery, 2018[12]b	New Zealand	73	60M, 13F	2.5–8 (5.2, mean)	Mean: 60 ± 24	Vitamin D3 + DHA	2000 IU/day	12 months	ABC	RDBPC
Mazahery, 2019[17]a	New Zealand	117	100M, 17F	2.5–8 (5.2, mean)	Mean: 63 ± 27	Vitamin D3	2000 IU/day	12 months	SRS, SPM	RDBPC
Mazahery, 2019[17]b	New Zealand	117	100M, 17F	2.5–8 (5.2, mean)	Mean: 56 ± 26	Vitamin D3 + DHA	2000 IU/day	12 months	SRS, SPM	RDBPC
Kerley, 2017	Ireland	38	33M, 5F	7.1 (mean)	Median: 54.2	Vitamin D3	2000 IU/day	5 months	ABC, DD-CGAS	RDBPC
RCTs not included in the meta-analysis but included in the overall interpretation ($n = 2$)										
Author, year	Country	Total	Sex distribution (M, F)	Age (years)	Baseline serum 25(OH)D (nmol/L)	Treatment	Dose	Duration	Outcome measure	Study quality
Azzam, 2014	Egypt	21	16 M, 5F	2–12	Mean: 59 ± 33	Vitamin D3	2000 IU/day	6 months	CARS, ATEC	Unclear randomization
Moradi, 2018	Iran	100	100 M	6–9 (7.6, mean)	Mean: 4.61 ± 12.6	Vitamin D3	300 IU/day/kg–5000 IU/day	4 months	SSGARS-2	Unclear randomization; placebo-controlled

Abbreviations: **ABC**, Aberrant Behavior Checklist; **CARS**, Childhood Autism Rating Scale; **ATEC**, Autism Treatment Evaluation Checklist; **SRS**, the Social Responsiveness Scale; **SPM**, Sensory Processing Measures; **DD-CGAS**, Developmental Disabilities-Children's Global Assessment Scale; **SSGARS-2**, the stereotypy subscale of the Gilliam Autism Rating Scale-Second Edition; **RDBPC**, Randomized Double-Blind Placebo-Controlled; **M**, male; **F**, female.

Repetitive and restricted behaviors and interests: RRB scores (measured by the ABC) did not differ between the vitamin D supplementation and placebo groups (pooled MD: 0.85; 95% CI: [−0.33, 2.02]; $p = 0.16$), and no heterogeneity was seen ($I^2 = 0\%$, $p = 0.77$) (Figure 3(c)). Removing the Mazahery, 2018b, study[12] that examined the effect of vitamin D/DHA together did not affect the overall result. Neither Mazahery et al.[17] (using the RRB sub-domain of the SRS) (Figure 4(c)) nor Moradi et al.[19] (using the SSGARS-2 subscale) showed an effect of vitamin D intervention on RRB ($p > 0.05$).

Effect of vitamin D on co-existing conditions

Hyperactivity: Based on three RCTs involving 104 participants, a significant difference was noted between the intervention and control groups in hyperactivity scores assessed by the ABC (pooled MD: −3.20; 95% CI: [−6.06, −0.34]; $p = 0.03$), and low heterogeneity was seen in the meta-analysis ($I^2 = 10\%$, $p = 0.33$) (Figure 5 (a)). Sensitivity analysis by removing the study[12] that tested the effect of vitamin D/DHA together had an effect on the overall result, and no statistically significant effect was found for vitamin D supplementation alone on ASD (pooled MD: −2.08; 95% CI: [−7.96, 3.80]; $p = 0.49$), and moderate heterogeneity was found ($I^2 = 54\%$, $p = 0.14$).

Irritability: Irritability scores (assessed by the ABC) did not differ between the vitamin D intervention and placebo groups (pooled MD: −2.31; 95% CI: [−6.08, 1.46]; $p = 0.23$), and substantial heterogeneity was found in the meta-analysis ($I^2 = 64\%$, $p = 0.06$) (Figure 5(b)). Sensitivity analysis by removing the study[12] that tested the effect of vitamin D/DHA together had no effect on the overall result.

Sensory issues: The Mazahery, 2019, study[17] was the only study that assessed the effect of vitamin D intervention on sensory issues (using the SPM sensory profile sub-domains of vision, hearing, touch, taste, and smell). Neither the vitamin D intervention group nor the placebo group showed any trends for improvement (all $p > 0.05$). Publication bias could not be determined for any outcome measures due to the small number of studies included the meta-analysis (n = 3).

Tolerability and safety

All RCTs included in this review concluded that vitamin D supplementation was well tolerated and safe. Adverse effects reported were not serious and were comparable across the intervention and control groups.

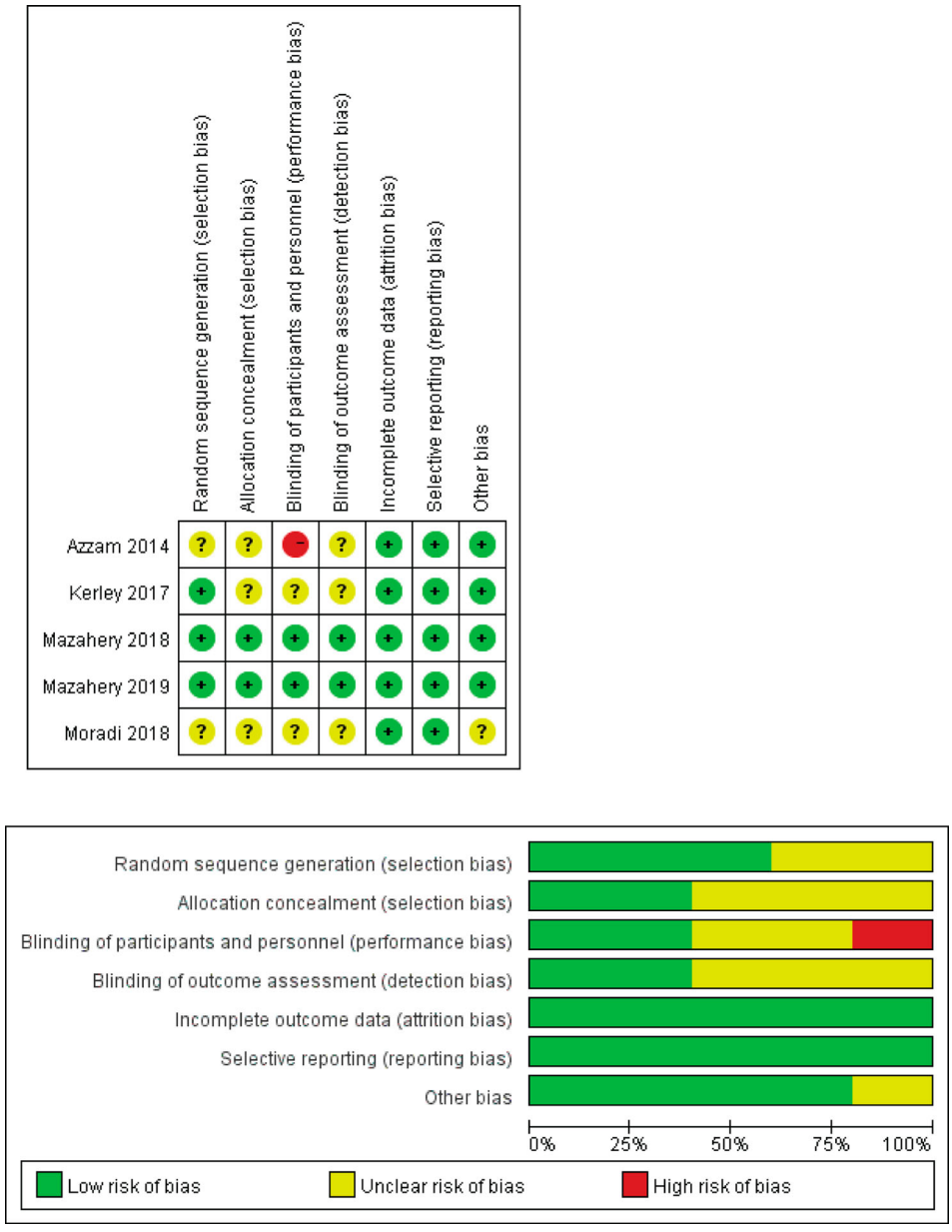

Figure 2. Risk for bias for individual and across studies.

Discussion

Only a few RCTs have been published on vitamin D supplementation for ASD, and only five trials were included in this review (three included in the meta-analysis and two in the overall interpretation), with a total of 349 participants. In contrast with the recent meta-analysis by Song *et al.*[14], our results indicated that there were no statistically significant differences between groups in terms of core symptoms and co-existing behaviors and conditions of ASD as measured by validated scales in this meta-analysis. With one exception, among the studies that used the ABC assessment tools there was a significant difference between groups for the hyperactivity subscale score. The limited available evidence suggests that vitamin D supplementation does not benefit the performance of children with ASD other than a significant improvement in hyperactivity symptoms.

Our findings should be considered cautiously because of possible influences of the different methodologies used in the included studies. First, most observational studies have shown that low vitamin D status in children at baseline is associated with the development of ASD[32,34,35], and a recent meta-analysis of the relationship between serum 25(OH)D levels and risk for ASD indicated an inverse association[36]. It has been proposed that oxidative stress and mitochondrial dysfunction are prevalent in individuals with ASD[37,38], whereas vitamin D has been

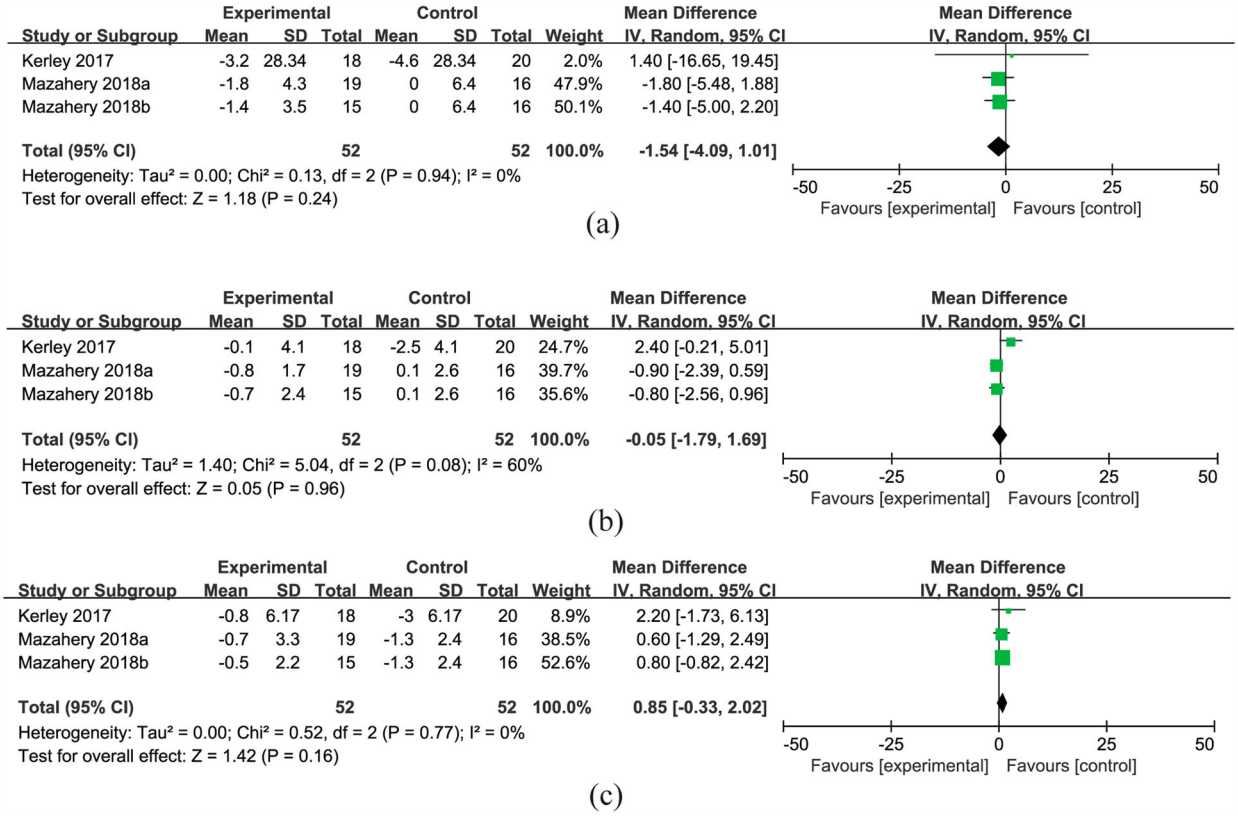

Figure 3. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation in children with ASD with placebo for social interaction (a), communication (b), and RRB (c) as assessed by the ABC measurement scale. Mazahery 2018a looked at the effect of receiving vitamin D supplementation only, while Mazahery 2018b looked at the effect of receiving vitamin D supplementation along with DHA.

shown to regulate cell proliferation and differentiation and to protect the genome from oxidative stress and DNA damage[39]. Participants with low serum 25(OH)D levels might therefore be expected to show the greatest effect of vitamin D supplementation on ASD. Gowda *et al.*[40] reported that the efficacy of vitamin D supplementation in improving serum 25(OH)D was mainly affected by baseline 25(OH)D levels, the length of supplementation, and habitual dietary intake of vitamin D. Those with lower baseline serum 25(OH)D levels had a greater chance of experiencing a greater increase in serum 25(OH)D in response to supplementation. However, most of the studies in the present meta-analysis included participants with sufficient 25(OH)D levels at baseline.

Second, two of the three RCTs included in the meta-analysis examined both vitamin D alone and vitamin D together with DHA supplementation[12,17]. Our sensitivity analysis, which excluded studies that administered vitamin D supplementation along with DHA, did not find any effect on the core symptoms of ASD. On the other hand, the results of combined vitamin D alone and vitamin D along with DHA supplementation on ASD showed a significant reduction in hyperactivity, which changed the effect of vitamin D-only supplementation on ASD. We also found no effect of vitamin D/DHA together on ASD (MD: −3.70; 95% CI: [−7.91, 0.51]), as shown in Figure 5(a). Additionally, the previous meta-analysis did not support an effect of DHA alone on ASD in children[41]. In this case, our analysis could not distinguish an independent effect of vitamin D on ASD. The loss of significance highlights the importance of combination therapy of vitamin D supplements on children with ASD. Whether vitamin D given together with DHA is more beneficial than vitamin D alone needs to be clarified in future RCTs.

Third, it may be possible that the measurement scales employed in the included studies influenced the findings. Although most of the included RCTs used the ABC to assess problem behaviors in ASD, other assessments designed for diagnostic purposes were only used in individual RCTs. Consequently, the generalizability of the results is limited. In addition, the inappropriate speech subscale of the ABC is not a comprehensive measure of communication compared to other assessment tools like the SRS, and therefore the results of the

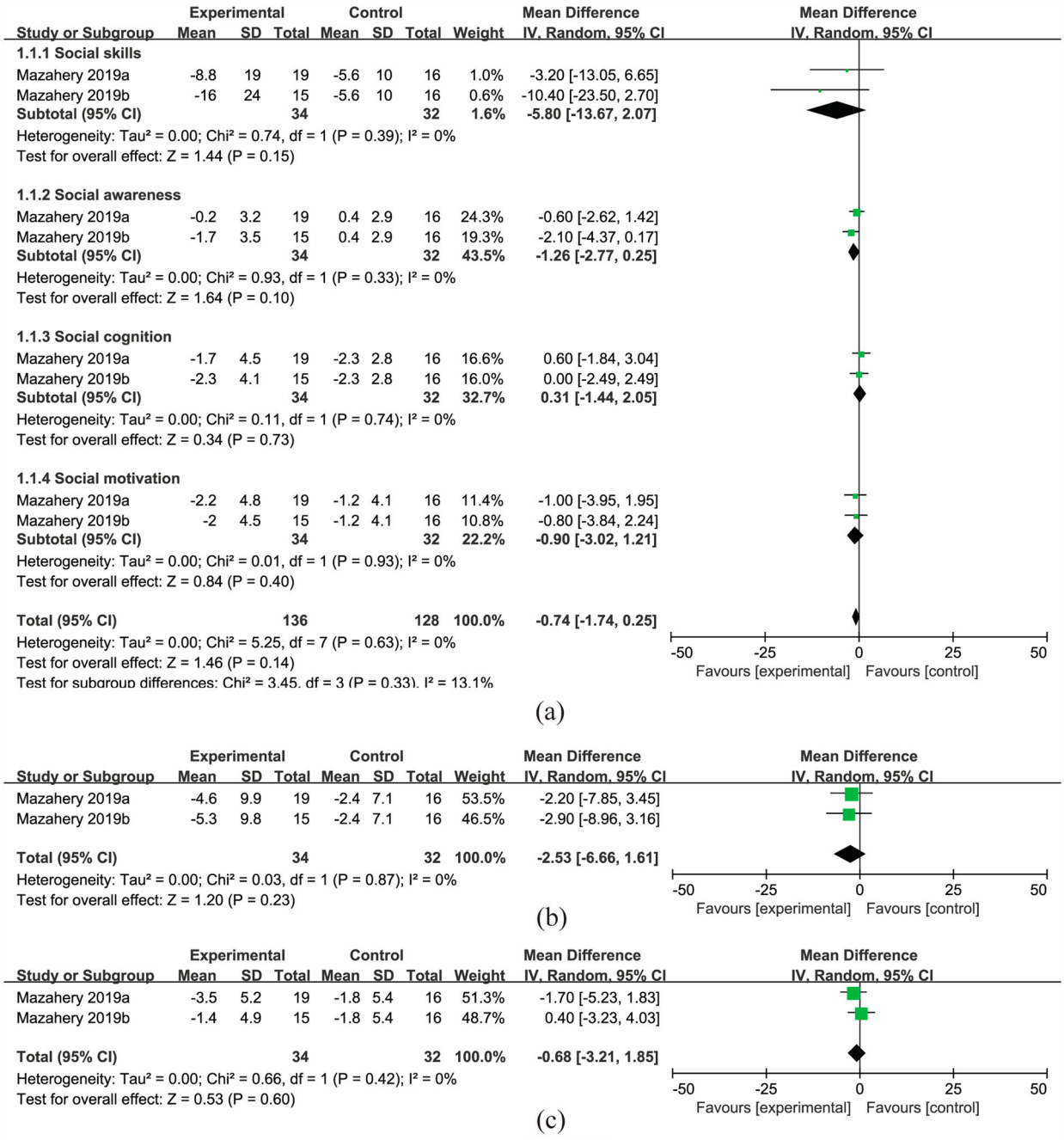

Figure 4. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation in children with ASD with placebo for social interaction (a), communication (b), and RRB (c) as assessed by the SRS measurement scale. Mazahery 2019a looked at the effect of receiving vitamin D supplementation only, while Mazahery 2019b looked at the effect of receiving vitamin D supplementation along with DHA.

communication subscale of the ABC should be interpreted with caution.

Our findings may have implications for future research. The current evidence does not support vitamin D supplementation as monotherapy for children with ASD. However, it seems feasible that vitamin D might be used to complement other treatments for ASD considering its long-term tolerability and acceptability[42,43], its critical role in brain function and development, and its roles in various metabolic pathways involved in the pathobiology of ASD[38]. It will be important to employ one uniform assessment tool in order to reduce methodological bias.

Strengths and limitations

There were several strengths in the present meta-analysis. First of all, it is the first systematic review to

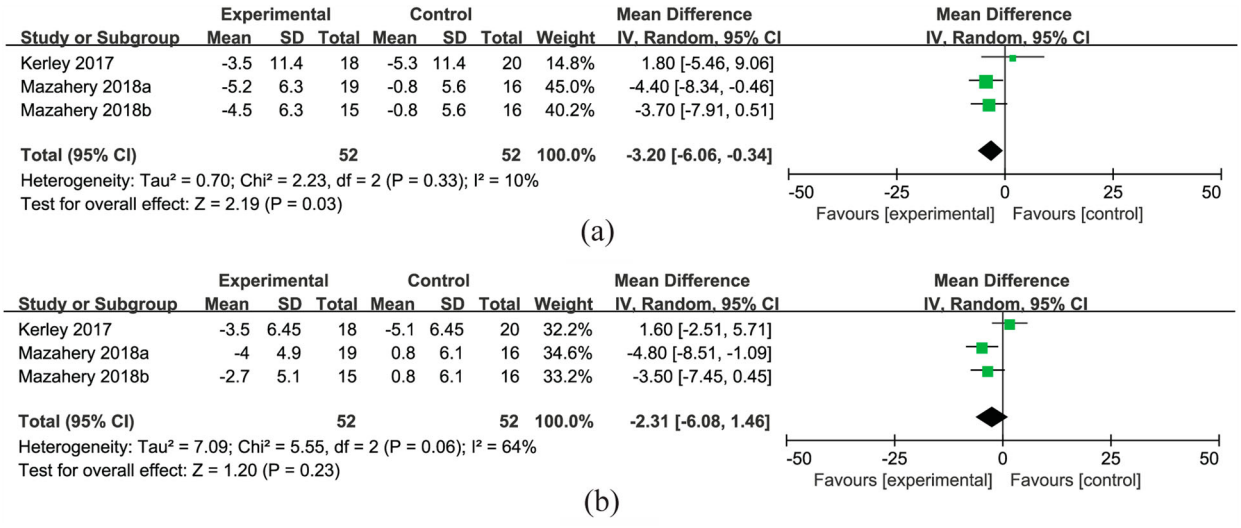

Figure 5. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation in children with ASD with placebo for hyperactivity (a) and irritability (b) as assessed by the ABC measurement scale. Mazahery 2018a looked at the effect of receiving vitamin D supplementation only, while Mazahery 2018b looked at the effect of receiving vitamin D supplementation along with DHA.

summarize the evidence regarding vitamin D supplementation for ASD treatment. We also applied to PROSPERO and registered the systematic review protocol prior to starting the review process (CRD42020157572). In addition, our meta-analysis used the rigorous methodology developed by the Cochrane Collaboration and applied several methods to reduce bias such as a comprehensive literature search and rigorous eligibility and methodological assessments. Lastly, two of the included studies were of high methodological quality, and heterogeneity among studies was rather low[12,17].

Based on only five RCTs with a limited number of 349 participants, our review is likely to be underpowered for addressing some outcomes and insufficient for drawing trustworthy conclusions. Furthermore, we note the small sample sizes in some trials, and there were potential biases across some of the study designs. Three studies had a high risk for selection bias due to uncertain allocation concealment and lack of blinding[13,18,19]. In addition, all three studies included in the meta-analysis were performed in high-income, developed countries, so the conclusions are not necessarily generalizable for low-income countries. Publication bias and subgroup analysis on important parameters such as trial duration and 25(OH)D levels at baseline could not be investigated through funnel plots or other analyses due to the limited number of studies.

To summarize, this study is the first systematic review to examine the available clinical data in order to assess the efficacy of vitamin D supplementation versus placebo for children with ASD. In the current meta-analysis, a small but significant benefit of vitamin D supplementation was found for hyperactivity, but our study did not support the evidence for vitamin D supplementation in improving core and other co-existing symptoms in children with ASD. Future RCTs that take low serum 25(OH) D levels at baseline, trial duration, and the dose and frequency of vitamin D supplementation into consideration are needed to determine the efficacy of vitamin D on ASD.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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