Review
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Effects of Vitamin D Supplementation on Children with Autism Spectrum Disorder: A Systematic Review and Meta-analysis

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The effect of vitamin D supplementation on individuals with autism spectrum disorder (ASD) is inconclusive. We aimed to conduct a meta-analysis of the available randomized controlled trials (RCTs) to explore whether vitamin D supplementation can improve core symptoms and coexisting conditions in children with ASD. Data were obtained by searching the PubMed, Embase, Web of Science, CINAHL and Cochrane Library databases up to February 2022 following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Using a random-effects model, mean differences with 95% confidence intervals (CIs) were calculated through a meta-analysis. There were eight RCTs with 266 children with ASD in the present review, among which six RCTs were included in the meta-analysis. Children who received vitamin D supplementation showed a significant improvement in stereotypical behavior scores (pooled mean difference (MD): −1.39; 95% CI: −2.7, −0.07; $p = 0.04$) with low heterogeneity ($I^2 = 34\%$), and there was a trend toward decreased total scores on the Social Responsiveness Scale (SRS) and Childhood Autism Rating Scale (CARS, $p = 0.05$); however, there were no other significant differences in the core symptoms of ASD and coexisting conditions between groups as measured by the Aberrant Behavior Checklist (ABC). Vitamin D supplementation appears to improve stereotypical behaviors but does not improve other core symptoms and coexisting conditions. Further randomized controlled trials with large sample sizes and individualized doses are needed.

KEY WORDS: Autism spectrum disorder; Core symptoms; Vitamin D intervention; Children; Meta-analysis.

INTRODUCTION

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by difficulties in social interaction, communication impairments, and repetitive and stereotyped behaviors [1,2]. According to previous studies, the prevalence rate of ASD has continuously increased, and no causal treatment is currently known [3]. Both genetic and environmental factors play a role in the etiology of ASD [4]. Among the environmental factors, vitamin D deficiencies are frequently reported in children with ASD [5-8]. A meta-analysis that included 870 children with ASD and 782 healthy controls concluded that children with ASD had significantly lower serum 25(OH)D levels than controls, suggesting that lower vitamin D levels might be a risk factor for ASD [9]. Additionally, vitamin D deficiency during pregnancy has been reported to contribute to the cause of autism [10-16], and vitamin D supplementation during pregnancy and early childhood might reduce the recurrence rate of ASD in newborn siblings [17]. A rat model study found that high-dose vitamin D showed both protective and treatment effects, with the protective effects being more robust than the treatment effects [18]. Many reported hy-

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potheses and possible mechanisms have been investigated to explain how vitamin D helps to protect and treat ASD [19-22]. For instance, activated vitamin D (calcitriol) can reduce the levels of inflammatory cytokines, which are increased in the brains of patients with ASD [23], and alleviate neuroinflammation [20]; calcitriol can elevate levels of the master antioxidant glutathione [19]; calcitriol can upregulate central serotonin levels through direct genetic regulation of tryptophan hydroxylase (TPH1 and TPH2), which are the rate-limiting enzymes of serotonin [21]; and vitamin D3 supplementation can increase neurite development (hippocampal cells), dopamine synthesis (rats), and nerve growth factor and neurotrophin-3 mRNA levels (astrocytes) [22]. These epidemiological and experimental studies have prompted several clinical trials to determine whether vitamin D supplementation can improve core symptoms in children with ASD [9-23]. In some case reports [24-26] and open-label trials [27], vitamin D supplementation was reported to effectively improve autism symptoms. However, the results of the randomized controlled trials (RCTs) are inconsistent. For example, Moradi *et al.* [28] showed that vitamin D supplementation was superior to a placebo in reducing the occurrence of stereotypical behavior; Mazahery *et al.* [29] showed a significant improvement in irritability and hyperactivity; and Javadfar *et al.* [30] showed significant alleviation of clinical symptoms as assessed by Childhood Autism Rating Scale (CARS), and the Autism Treatment Evaluation checklist (ATEC). Kerley *et al.* [31] reported that vitamin D supplementation had no effect on the primary outcomes of children with ASD. Two recently published meta-analyses compared some of these studies. One meta-analysis by Song *et al.* [32] compared the total Social Responsiveness Scale (SRS) and CARS scores but did not compare the core symptom-related subscale scores. Another meta-analysis by Li *et al.* [33] combined the vitamin D plus DHA supplementation group in the meta-analysis.

Given the inconsistent conclusions from the available RCTs and the importance of clarifying the role of vitamin D supplementation in ASD treatment, a meta-analysis on this subject is warranted. This study performed a meta-analysis of the available RCTs to measure whether 25(OH)D levels increased after vitamin D supplementation and whether core symptoms and coexisting conditions improved after improvements in vitamin D status in children with ASD and healthy controls.

MATERIALS AND METHODS

Search Strategy

We conducted this review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [34] and formulated it following the Population, Intervention, Comparison, Outcome and Study design (PICOS) criteria [35]. Electronic databases, including the PubMed, Embase, Cochrane Library, Web of Science, and CINAHL databases, were searched up to February 2022. Different search strategies were adopted for different electronic databases. The search terms for PubMed were as follows: ("Autistic Disorder"[MeSH Terms] OR ("kanner's syndrome"[Title/Abstract] OR "Autism"[Title/Abstract] OR "ASD"[Title/Abstract] OR "autism spectrum disorders"[Title/Abstract])) AND ("vitamin d"[MeSH Terms] OR "ergocalciferols" [Title/Abstract] OR "cholecalciferols"[Title/Abstract] OR "vitamin d2"[Title/Abstract] OR "vitamin d3"[Title/Abstract] OR "25 hydroxyvitamin d"[Title/Abstract] OR "25-Hydroxycholecalciferol"[Title/Abstract] OR "25 hydroxyvitamin d3"[Title/Abstract] OR "25 hydroxyvitamin d2"[Title/Abstract]) AND ("supplementation"[Title/Abstract] OR "supplements"[Title/Abstract] OR "intake"[Title/Abstract] OR "Intervention"[Title/Abstract] OR "treatment"[Title/Abstract]) AND ("randomized controlled trial"[Publication Type] OR "controlled clinical trial"[Publication Type] OR "randomized"[Title/Abstract] OR "randomly"[Title/Abstract] OR "trial"[Title/Abstract]). The specific search strategies for the Cochrane library and Embase are presented in the Supplementary Figures 1, 2 (available online). Registration number: CRD42022307625.

Inclusion and Exclusion Criteria

Two reviewers (MZ and YRW) independently screened the retrieved references for inclusion based on titles, keywords and abstracts in duplicate. Articles for which the titles, keywords and abstracts did not provide sufficient information for a decision on selection were reviewed as full texts. Disagreements during the study selection process were resolved by discussion and with the involvement of a third review author (ZXL) until consensus was achieved. Studies were selected if they fulfilled the following inclusion criteria: (1) the study was a randomized double-blind placebo-controlled clinical trial; (2) the studied subjects were children aged up to 18 years with an ASD diagnosis based on the established criteria; (3) the inter-

vention protocols were specified for vitamin D supplementation only in children with ASD and placebos were used in the control group; and (4) the study used at least one outcome measurement scale. Studies were excluded if they were unpublished or ongoing studies, duplicate articles, case reports, conference articles, letters, animal studies, retracted articles, reviews or meta-analyses, or were not written in English or Chinese.

Data Extraction and Quality Assessment

Two reviewers (MZ and YRW) independently extracted the following data from each study in duplicate: the first author's name, year of publication, research country, sexes and ages of participants, dose and duration of the intervention, serum 25(OH)D levels, changes in autism assessment scores and information about study quality. The Cochrane Risk Assessment Tool and the Cochrane bias test were used to evaluate the quality of the randomized controlled trials [36].

Statistical Analysis

Mean changes in the outcome scores from baseline to endpoint and their standard deviations (SDs) were entered and analyzed using Review Manager version 5.4 (Cochrane Collaboration), and mean differences (MDs) with 95% confidence intervals (CIs) between the vitamin D and placebo groups were calculated. If only baseline and endpoint data were available, changes were calculated by subtracting the baseline value from the endpoint value, and the SD was imputed using the p values resulting from the paired t test. If the p values resulting from the paired t test were not available, the mean correlation coefficient for an outcome from another RCT in the meta-analysis was used to impute the SD. Random effects were used in the primary meta-analysis, and heterogeneity between included studies was evaluated by both the Q-statistic ($p <$ 0.1 showed considerable heterogeneity) and the I^2 index (I^2 0−40%, low; 30−60%, moderate; 50−90%, substantial; 75−100%, considerable heterogeneity) [37].

RESULTS

Search Results and Study Selection

A total of 445 articles were identified in our initial search, and 83 were duplicates. Among the 445 studies, 322 were from Embase, 28 were from PubMed, 48 were from Web of Science, 24 were from the Cochrane Library, 22 were from CINAHL and 1 was from Wanfang. After the removal of duplicates, 348 articles were excluded by screening titles or abstracts, leaving 14 studies to be assessed for eligibility by reading the full texts. Among the 14 full texts, 6 were excluded (4 were not RCTs, and 2 did not use the correct interventions), and 8 studies met the inclusion criteria. Among the eight studies, three were series studies conducted by the same team [29,38,39]. The subjects of the study published in 2020 [39] were all investigated the study published in 2019 [38], and the SRS was used in both studies; thus, the study published in 2020 was not included in the final meta-analysis. One study was reported to have a high risk of other bias [40], and it was not included in the meta-analysis. The screening and selection processes are presented in the flowchart in Figure 1.

Study Characteristics

The main characteristics of the selected studies are illustrated in Table 1. Among the 8 selected studies, three were conducted in New Zealand [29,38,39], three were conducted in Iran [28,30,41], one was conducted in Ireland [31] and one was conducted in China [40]. Six studies recruited both male and female participants, and two studies recruited males only. The ages of the recruited children ranged from 2.5−14 years. The vitamin D intervention was in the form of vitamin D3 in 6 RCTs, and there was no specific description of the vitamin D type in the other 2 studies. Intervention doses ranging from 800 IU/day to 50,000 IU/week and 2,000 IU/day were used in half of the RCTs. The duration of the interventions ranged from 10 weeks to 12 months.

The outcome measurement scales used in the included studies were the Aberrant Behavior Checklist (ABC), the SRS, the CARS, the ATEC, the Sensory Processing Measure (SPM), the Gilliam Autism Rating Scale-Second Edition (GARS-2) and the Developmental Disabilities-Children's Global Assessment Scale (DD-CGAS). Each scale included several subscales and the corresponding scores.

Risk of Bias in the Included RCTs

Figure 2 shows the risk of bias in the included RCTs. Three studies that did not mention the random-sequence generation method were considered to have an unclear risk of randomization bias [28,40,41]. Four studies were

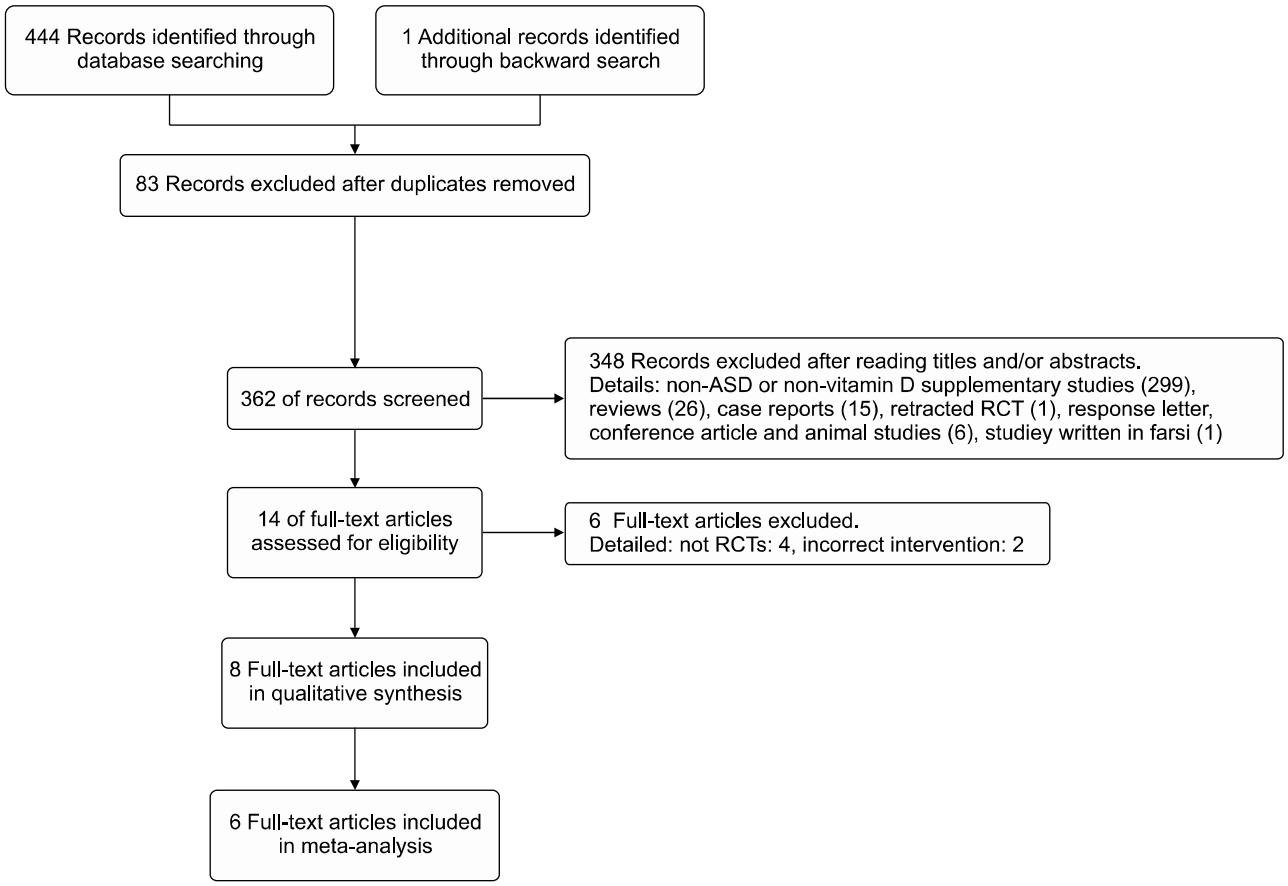


Fig. 1. Flow chart illustrating the literature search process.
RCTs, randomized controlled trials.

considered to have an unclear risk of allocation concealment bias owing to insufficient information [28,31,40,41]. Three studies were assessed as having an unclear risk of performance bias and detection bias due to an insufficient description of double blinding (researchers and children/caregivers) and no details were provided for outcome assessment blinding [28,31,41]. Two studies were considered to have an unclear risk of attrition bias due to a lack of description about the loss to follow-up [39,41]. One study was considered to have a high risk of other bias [40].

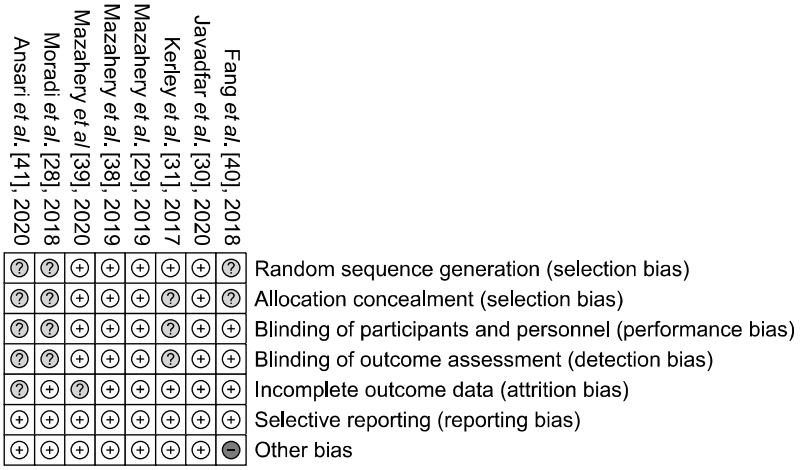
Serum 25(OH)D Concentrations after Vitamin D Supplementation

Serum 25(OH)D concentrations were evaluated at baseline and at the endpoint of vitamin D supplementation using ELISA, HPLC, and LC–MS/MS methods, and detailed 25(OH)D levels were provided in 4 RCTs (Table 1). Both baseline and final 25(OH)D levels were used as the outcome index because there was insufficient information for estimating the SDs of the changes. There were no significant differences in baseline 25(OH)D levels between the intervention and placebo groups in the four included studies (pooled MD: 0.2; 95% CI: −1.70, 2.10; $p = 0.84$, Fig. 3A), and no heterogeneity was observed ($I^2 = 0\%$). As shown in Figure 3B, the total effect amount fell on the right side of the invalid line, and a significant difference was observed in the endpoint 25(OH)D levels (pooled MD: 16.81; 95% CI: 11.89, 21.72; $p < 0.00001$) with substantial heterogeneity ($I^2 = 76\%$, $p = 0.005$). Removing one of the studies [41] remarkably improved the heterogeneity, as I^2 decreased from 76% to 0%. After removing the study, the overall result was unchanged, with a p value that was still less than 0.00001. This finding illustrated that 25(OH)D levels were significantly increased after vitamin D intervention. Thus, vitamin D supplementation can improve vitamin D nutritional status in children with ASD.

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

Study	Country	Number	Age, mean (yr)	Sex, M/F	Dose	Duration	Baseline 25-OH-VD level, mean	Final 25-OH-VD level, mean	Outcome measure	Quality
RCTs included in the Meta-analysis (n = 6)										
Kerley *et al.* [31], 2017	Ireland	18 (VD) 20 (placebo)	7.9 (VD) 6.9 (placebo)	15/3 (VD) 18/2 (placebo)	2,000 IU/d (vitamin D3)	20 weeks	58.60 (nmol/L, VD) 51.70 (nmol/L, placebo)	86.10 (nmol/L, VD) 50.60 (nmol/L, placebo)	ABC, SRS, DD-CGAS	RDBPC
Mazahery *et al.* [29], 2019	New Zealand	19 (VD) 16 (placebo)	5.3 (VD) 5.7 (placebo)	16/3 (VD) 13/3 (placebo)	2,000 IU/d (vitamin D3)	12 months	68.00 (nmol/L, VD) 55.00 (nmol/L, placebo)	95.00 (nmol/L, VD) NA (placebo)	ABC	RDBPC
Mazahery *et al.* [38], 2019	New Zealand	19 (VD) 16 (placebo)	2.5−8 (5.2)	NA	2,000 IU/d (vitamin D3)	12 months	63.00 (nmol/L, VD) 56.00 (nmol/L, placebo)	NA	SRS, SPM	RDBPC
Javadfar *et al.* [30], 2020	Iran	22 (VD) 21 (placebo)	8.88 (VD) 8.95 (placebo)	20/2 (VD) 16/5 (placebo)	300 IU/kg, maximum of 6,000 IU/d (unclear vitamin D3 OR D2)	15 weeks	8.19 (ng/ml, VD) 10.84 (ng/ml, placebo)	39.10 (ng/ml, VD) 8.94 (ng/ml, placebo)	ABC, CARS, ATEC	RDBPC
Moradi *et al.* [28], 2018	Iran	25 (VD) 25 (placebo)	7.62	25/0 (VD) 25/0 (placebo)	300 IU/kg, maximum of 5,000 IU/d (vitamin D3)	3 months	12.60 (ng/ml, VD) 11.52 (ng/ml, placebo)	24.36 (ng/ml, VD) 11.08 (ng/ml, placebo)	GARS-2	RDBPC
Ansari *et al.* [41], 2020	Iran	10 (VD) 10 (placebo)	6−14	10/0 (VD) 10/0 (placebo)	50,000 IU/week or 50,000 IU/2 weeks (vitamin D3)	10 weeks	11.12 (ng/ml, VD) 10.30 (ng/ml, placebo)	31.60 (ng/ml, VD) 10.20 (ng/ml, placebo)	GARS-2	RDBPC
RCTs not included in the meta-analysis (n = 2)										
Fang *et al.* [40], 2018	China	12 (VD) 12 (placebo)	10.4 (VD) 10.2 (placebo)	8/4 (VD) 7/5 (placebo)	800 IU/d (unclear vitamin D3 OR D2)	12 months	31.8 (nmol/L, VD) 28.3 (nmol/L, placebo)	NA	CARS	RCT
Mazahery *et al.* [39], 2020	New Zealand	15 (VD) 16 (placebo)	5.3	NA	2,000 IU/d (vitamin D3)	12 months	NA	NA	SRS	RDBPC

RCTs, randomized controlled trials; VD, vitamin D supplementation; RDBPC, randomized double-blind placebo-controlled clinical trial; ABC, Aberrant Behavior Checklist; SRS, Social Responsiveness Scale; DD-CGAS, Disabilities-Children's Global Assessment Scale; CARS, Childhood Autism Rating Scale; GARS-2, Gilliam Autism Rating Scale-Second Edition; SPM, Sensory Processing Measure; ATEC, Autism Treatment Evaluation checklist; NA, not available.



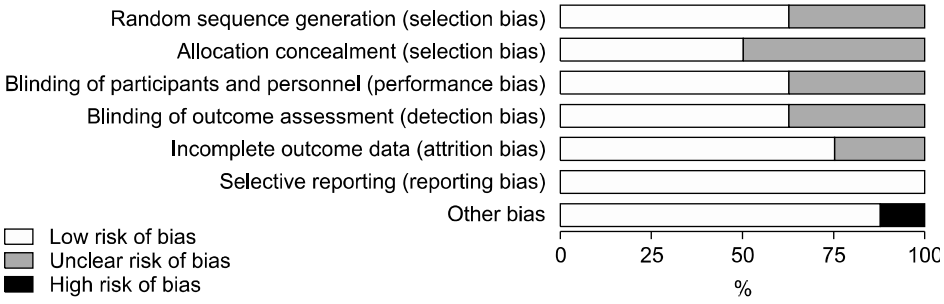
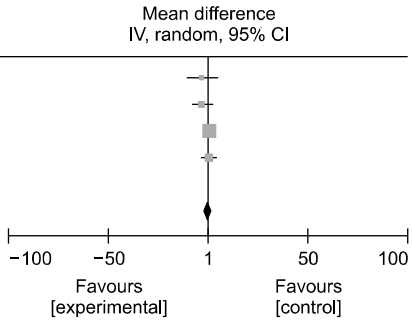


Fig. 2. Risk of bias of the included studies.

A

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Javadfar *et al.* [30], 2020	8.19	6.78	22	10.84	16.8	21	6.1%	−2.65 [−10.37, 5.07]
Kerley *et al.* [31], 2017	20.68	7.92	18	23.36	7.16	20	15.6%	−2.68 [−7.50, 2.14]
Moradi *et al.* [28], 2018	12.6	4.61	25	11.52	4.87	25	52.3%	1.08 [−1.55, 3.71]
Ansari *et al.* [41], 2020	11.12	4.29	10	10.3	4.21	10	26.1%	0.82 [−2.91, 4.55]
Total (95% CI)			75			76	100.0%	0.20 [−1.70, 2.10]

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 2.43$, df = 3 ($p = 0.49$); $I^2 = 0\%$
Test for overall effect: Z = 0.21 ($p = 0.84$)



B

Study or subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean difference IV, random, 95% CI
Javadfar *et al.* [30], 2020	30.91	33.71	22	8.94	8.03	21	8.8%	21.97 [7.47, 36.47]
Kerley *et al.* [31], 2017	34.44	7.16	18	7.92	7.92	20	27.5%	14.20 [9.41, 18.99]
Moradi *et al.* [28], 2018	24.36	6.48	25	3.95	3.95	25	32.8%	13.28 [10.31, 16.25]
Ansari *et al.* [41], 2020	31.6	4.45	10	3.88	3.88	10	30.9%	21.40 [17.74, 25.06]
Total (95% CI)			75			76	100.0%	16.81 [11.89, 21.72]

Heterogeneity: $Tau^2 = 16.89$; $Chi^2 = 12.74$, df = 3 ($p = 0.005$); $I^2 = 76\%$
Test for overall effect: Z = 6.70 ($p < 0.00001$)

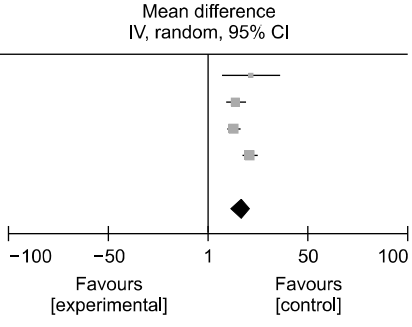


Fig. 3. Forest plot of the random-effects meta-analysis comparing baseline (A) and final (B) 25(OH)D levels in the vitamin D supplementation group and the placebo group.
SD, standard deviation; CI, confidence interval; IV, inverse variance.

Effect of Vitamin D Supplementation on Core Symptoms and Coexisting Conditions

Pooled effect assessed by the SRS

Among the six included studies, the total SRS scores were available in two RCTs; ABC scores, including subscale scores, were available in three RCTs; and stereotypy subscale scores of the GARS-2 were available in two RCTs. Figure 4 shows the forest plot of the random-effects meta-analysis comparing vitamin D supplementation in children with ASD with a placebo as assessed by the total SRS score. The total effect amount fell on the left side of the invalid line for changes in the mean scores (pooled MD: −8.74; 95% CI: −17.45, −0.03; $p = 0.05$), and no heterogeneity was observed ($I^2 = 0\%$). Since the p value was equal to 0.05, the pooled effect did not differ between the intervention and placebo groups.

Pooled effect assessed by the ABC and GARS-2

Social interaction/lethargy: As illustrated in Figure 5A, the pooled effect estimates for social interaction did not differ between the intervention and placebo groups in changes in the mean scores (pooled MD: −0.07; 95% CI: −1.70, 1.57; $p = 0.93$), and no heterogeneity was observed ($I^2 = 0\%$). Other measurement tools, including the DD-CGAS and the social interaction subdomain of the SPM, were used in two of the included RCTs [31,38], and no effect of vitamin D supplementation on social interaction was observed in studies using the DD-CGAS and SPM.

Restrictive and repetitive behaviors (RRBs) and stereotypical behaviors: There were no significant differences in RRBs between the intervention and placebo groups as assessed by changes in the mean scores of the ABC (pooled MD: −0.05; 95% CI: −1.19, 1.10; $p = 0.93$), and low heterogeneity was observed ($I^2 = 33\%$) (Fig. 5B). Similarly, Mazahery *et al.* [38], who used the RRB subdomain of the SRS, did not observe any treatment effect of vitamin D supplementation on RRBs. However, the opposite result was observed in changes in the mean scores assessed by the stereotypy subscale of the GARS-2. Based on the two included RCTs involving 70 participants [28,41], a significant difference was observed between the intervention and placebo groups (pooled MD: −1.39; 95% CI: −2.7, −0.07; $p = 0.04$), and low heterogeneity was observed ($I^2 = 34\%$) (Fig. 6).

Communication/inappropriate speech: The communication scores did not differ between the intervention and placebo groups in changes in the mean scores (pooled MD: −0.04; 95% CI: −1.19, 1.10; $p = 0.94$), and moderate heterogeneity was observed ($I^2 = 57\%$); details are shown in Figure 5C. Similarly, Mazahery *et al.* [38] and Kerley *et al.* [31], who used the communication subdomains of the SRS and DD-CGAS, did not observe any treatment effect of vitamin D supplementation on communication.

Irritability: Based on the three included RCTs involving 116 participants, no difference was noted between the intervention and control groups in irritability scores assessed by the ABC (pooled MD: −1.79; 95% CI: −4.42, −0.85; $p = 0.18$), and moderate heterogeneity was observed in the meta-analysis ($I^2 = 61\%$, $p = 0.08$) (Fig. 5D). In the sensitivity analysis, the removal of any one of the three included studies had no effect on the overall result.

Hyperactivity: Hyperactivity scores did not differ between the intervention and placebo groups in changes in the mean scores (pooled MD: −1.35; 95% CI: −4.37, 1.67; $p = 038$), and low heterogeneity was observed ($I^2 = 48\%$, $p = 0.14$) (Fig. 5E).

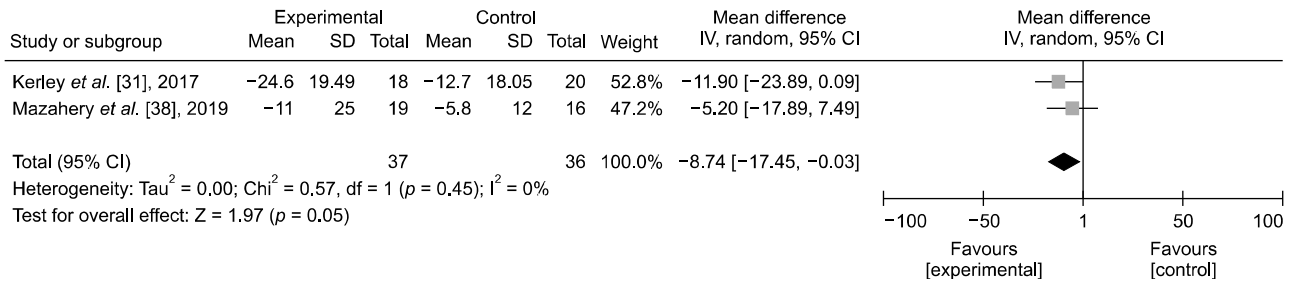


Fig. 4. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation with placebo treatment for core symptoms in children with ASD, as assessed by the SRS.
ASD, autism spectrum disorder; SRS, Social Responsiveness Scale; SD, standard deviation; CI, confidence interval; IV, inverse variance.

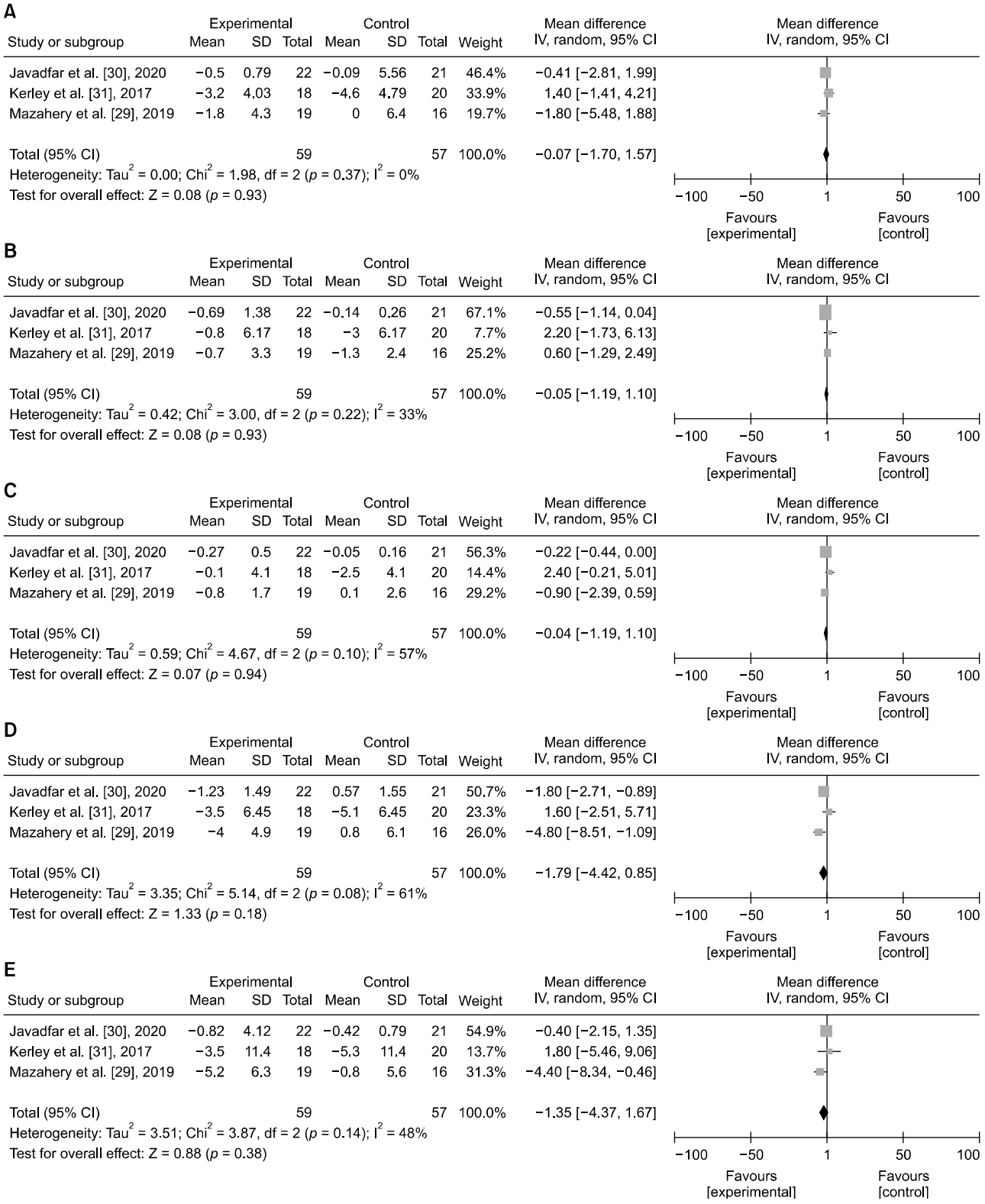


Fig. 5. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation with placebo treatment for lethargy (A), stereotypical behavior (B), inappropriate speech (C), irritability (D) and hyperactivity (E) in children with ASD, as assessed by the ABC.
ASD, autism spectrum disorder; ABC, Aberrant Behavior Checklist; SD, standard deviation; CI, confidence interval; IV, inverse variance.

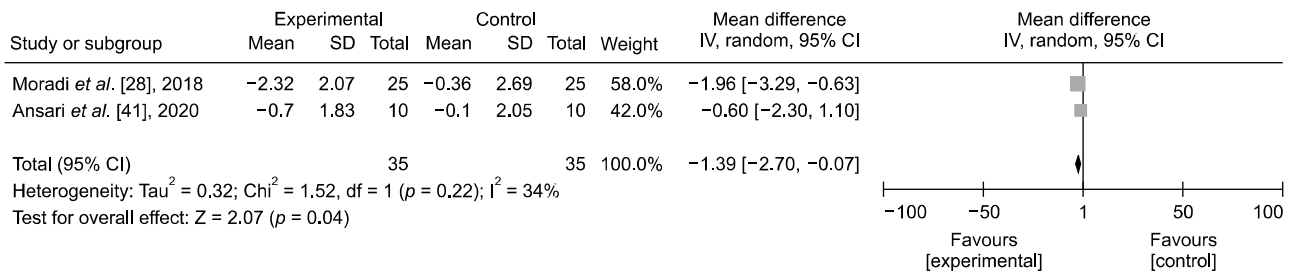


Fig. 6. Forest plot of the random-effects meta-analysis comparing vitamin D supplementation with placebo treatment for stereotypy in children with ASD, as assessed by the stereotypy subscale of the GARS-2.
ASD, autism spectrum disorder; GARS-2, Gilliam Autism Rating Scale-Second Edition; SD, standard deviation; CI, confidence interval; IV, inverse variance.

DISCUSSION

There have been eight RCT reports published on vitamin D supplementation for individuals with ASD; among them, three were series studies that used the same group of participants [29,38,39]. Since different measurement scales were used, two of the three were included in the meta-analysis [29,38]. Another study that was not included was reported to have a high risk of other bias [40]. Based on the 6 included RCTs with a total of 176 participants, no statistically significant differences were found between vitamin D intervention and placebo groups in terms of core symptoms and coexisting conditions as measured by the ABC ($p > 0.05$) and SRS ($p = 0.05$) in this meta-analysis. This result is basically consistent with one of the recently published meta-analyses by Li *et al.* [33]. Among the studies that used the GARS-2, there was a significant difference in stereotyped behaviors between groups.

Two recent meta-analyses reported the effect of vitamin D supplementation in the treatment of children with ASD. Song *et al.* [32] used the postintervention total SRS and CARS scores as the outcome index, and they found that the outcome scores in the experimental group were significantly improved compared with those in the placebo group ($p = 0.03$). According to the Cochrane handbook for systematic reviews of interventions, a comparison of the change values is better than a comparison of the postintervention values because the former has higher statistical power and efficiency. In addition, no core symptom-related subscale outcomes, such as social interaction, communication, and RRBs, were compared in Song *et al.*'s [32] study. Thus, even if the total scores improve, whether the core symptoms have improved is still unclear. The other meta-analysis by Li *et al.* [42] used the change values rather than the endpoint values, and they compared the subscale scores of the ABC and SRS. They found that there was no significant difference in ASD core symptoms and coexisting behaviors between groups, except for hyperactivity ($p = 0.03$). Vitamin D supplementation groups were combined with DHA supplementation groups in the final meta-analysis in Li *et al.*'s [42] study because there were only 2 RCTs available. The present review was the first meta-analysis comparing change values to measure the effect of single vitamin D supplementation on core symptoms and coexisting conditions in children with ASD. There were also limitations in the present meta-analysis. Since only eight RCTs with a limited number of 266 participants were reviewed and analyzed, our meta-analysis was likely to be underpowered for obtaining robust conclusions.

The doses and duration of vitamin D supplementation and endpoint serum 25(OH)D levels may be factors affecting interventional effects. Among the included RCTs, two trials administered vitamin D at a dose per kilogram of body weight [28,30], one trial administered vitamin D at a relatively high dose (50,000 IU/week) [41], and the others administered low doses (800−2,000 IU/d). The maximum total dose was less than 730,000 IU, and this dose of vitamin D supplementation significantly improved the nutritional status of 25(OH)D in children with ASD with no evidence of toxicity. According to the Endocrine Society of the United States, the ideal serum 25(OH)D level is 100−150 nmol/L (40−60 ng/ml) [43]. Greater improvement in ASD rating scale scores was observed in children with endpoint 25(OH)D levels > 100 nmol/L (40 ng/ml) than in those with endpoint levels ≤ 100 nmol/L (40 ng/ml) [26]. The endpoint 25(OH)D levels after treatment were not up to 100 nmol/L (40 ng/ml) in all of the included trials. Low endpoint 25(OH)D levels might be the

reason vitamin D supplementation did not benefit children in these trials, and relatively high dose and high endpoint 25(OH)D levels, for example, levels above 100 nmol/L (40 ng/ml), might be required in ASD treatment. A total of 11.11% (2/18) of the children with ASD exhibited decreased or unchanged 25(OH)D levels after vitamin D supplementation in Kerley *et al.*'s [44] trial. The rates of increased serum 25(OH)D levels in patients with ASD were reported to be significantly lower than those in asthmatic children, indicating that altered absorption or metabolism might exist. In patients with severe vitamin D absorption or metabolic disorders, calcitriol supplementation may be considered. In brief, serum 25-OHD levels should be assessed before, during and after treatment, combined with body weight, to determine the vitamin D dosage and target for a final 25-OHD level of at least 100 nmol/L (40 ng/ml).

Age at the start of treatment is another factor that needs to be considered; it has been reported that reductions in total CARS and ABC scores were more pronounced in younger children with ASD (under 3 years old) after vitamin D supplementation [45]. Most participants in the included trials were older than 5 years, and it is possible that less neuroprotection and benefits might be obtained from vitamin D supplementation because neuronal networks in these participants were already established, suggesting that vitamin D supplementation should be initiated as early as possible.

Vitamin D is inexpensive and safe. Feng *et al.* [45] used a relatively high dose (150,000 IU/month, intramuscularly administered and 400 IU/day and orally administered for 3 months), and no overdose or toxic reactions were observed. The low cost and high benefit-to-risk ratio of vitamin D supplementation make it the most promising treatment for autism. Considering that only a few RCTs have been published, more trials are desperately needed. The limitations of the included trials will provide directions for improvement in future research. We summarized the limitations and listed them as follows: 45% (9/20) of the samples in the placebo group showed increased 25(OH)D levels after placebo administration (+1 to +45 nmol/L) [31]; fixed and low vitamin D dosing was used; and the enrolled subjects were older.

In conclusion, children who received vitamin D supplementation showed a small but significant improvement in stereotypical behaviors but did not show improvements in other core symptoms and coexisting conditions. Further randomized controlled double-blind trials with large sample sizes are needed, and data on background diet, sun exposure, baseline and endpoint 25(OH)D levels, age, and intervention dose and duration should be considered.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conceptualization: Min Zhang, YiRan Wu. Data curation: Min Zhang, YiRan Wu, ZhaoXu Lu, MeiYan Song. Formal analysis and writing (original draft): Min Zhang, YiRan Wu. Methodology: ZhaoXu Lu, MeiYan Song, XiaoLan Huang, LaLa Mi. Review and editing: XiaoLan Huang, LaLa Mi, Jian Yang, Xiaodai Cui. Project administration: Jian Yang, Xiaodai Cui. Supervision: Jian Yang, Xiaodai Cui.

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