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## Lack of Effect of Vitamin D3 Supplementation in Autism: a 20-week, Placebo-Controlled RCT

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# Lack of effect of vitamin D<sub>3</sub> supplementation in autism: a 20-week, placebo-controlled RCT

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## ABSTRACT

**Objectives** Data suggest a potential role for vitamin D in autism spectrum disorder (ASD). We wanted to assess the effect of vitamin D<sub>3</sub> supplementation compared with placebo in children with ASD.

**Design** This was a double-blind, randomised, placebo-controlled trial.

**Setting** A paediatric outpatient centre at high latitude over the winter season in Dublin, Ireland (53°N).

**Patients** 42 children with ASD.

**Interventions** 2000 IU vitamin D<sub>3</sub> supplementation or placebo daily for 20 weeks.

**Main outcome measures** Assessments were completed at baseline and after 20 weeks of supplementation. The primary outcome was the stereotypic behaviour subscale from the Aberrant Behaviour Checklist (ABC). Secondary exploratory outcomes included additional subscales from the ABC, the Social Responsiveness Scale and rating on the Developmental Disabilities—Children's Global Assessment Scale (DD-CGAS) as well as biochemical parameters of total vitamin D status (25-hydroxyvitamin D (25(OH)D)), immunity and systemic inflammation.

**Results** 38 children completed the trial. Baseline 25(OH)D was 54.2±19.7 nmol/L. Following vitamin D<sub>3</sub> supplementation, there was a significant increase in 25(OH)D to 83.8 nmol/L (p=0.0016) but no effect on the primary endpoint. However, there was an improvement in self-care on DD-CGAS (p=0.02). In contrast, there was also a trend toward decreased inappropriate speech in the placebo group (p=0.08).

**Conclusion** Vitamin D supplementation had no effect on the primary outcome with limited and inconsistent effects in children with ASD. Considering the other promising data as well as the relative safety and cheapness of vitamin D supplementation, further trials are warranted.

**Trial registration** NCT02508922.

## INTRODUCTION

Autism spectrum disorder (ASD), a complex, heterogeneous neurodevelopmental disorder, has an estimated prevalence of ~1% in children. ASD is associated with significant genetic risk factors. However, the impact of environmental factors cannot be discounted. Indeed, epigenetic mechanisms have been implicated suggesting that gene-environment interactions are important in mediating risk.<sup>1,2</sup>

One potential environmental risk factor for ASD is vitamin D deficiency (VDD) which affects ~1 billion people.<sup>3</sup> While VDD has detrimental effects on bone health, recent reports

## What is already known on this topic?

- ▶ Autistic children are at risk for low vitamin D intakes and status.
- ▶ Vitamin D is hypothesised to have neurological, immunomodulatory and anti-inflammatory roles, which may be relevant in autism.
- ▶ Recent animal model and open-label studies as well as a double-blind, randomised, placebo-controlled trial suggest benefit of vitamin D supplementation.

## What this study adds?

- ▶ We present one of first randomised controlled trials of vitamin D supplementation in autism.
- ▶ Vitamin D status increased significantly, but there was no effect on stereotypical behaviour as assessed with Aberrant Behaviour Checklist (primary endpoint).
- ▶ We did observe some benefits, but this was inconsistent and further trials are recommended.

suggest implications for neurological, immune and inflammatory disorders due to its unique role in brain homeostasis, neurodevelopmental and immunological modulation and gene regulation.<sup>4-7</sup>

Multiple studies have noted inadequate vitamin D intake<sup>8-11</sup> and/or status<sup>11-21</sup> in children with ASD. Indeed, a 2016 meta-analysis of 11 studies, including 870 ASD children and 782 typically developing children, demonstrated significantly lower 25-hydroxyvitamin D (25(OH)D) in ASD compared with controls.<sup>22</sup>

Recent reviews have detailed the associations between vitamin D and ASD.<sup>4-7, 23</sup> VDD has been implicated in the development of ASD symptoms in an animal model.<sup>24</sup> Low-level evidence based on a single case report<sup>25</sup> and two open-label studies support the role of vitamin D supplementation in ASD.<sup>26, 27</sup> Additionally, a single, double-blind, randomised, placebo-controlled trial reported benefit of supplementation in ASD.<sup>28</sup> However, any potential mechanisms mediating a positive effect of vitamin D supplementation in ASD remain obscure. Therefore, we wanted to rigorously assess the effects of vitamin D<sub>3</sub> supplementation in ASD.

## METHODS

This trial was conducted at the National Children's Hospital (Dublin, Ireland) following institutional



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review board approval. At baseline, trial information was provided, and written parental/guardian consent was obtained prior to study procedures.

### Subjects

ASD children were recruited from paediatric neurodevelopmental and general outpatient clinics as well as through community ASD services. Inclusion criteria included clinician-diagnosed ASD (by either Autism Diagnostic Observation Schedule, Diagnostic and Statistical Manual of Mental Disorders or Diagnostic Instrument for Social and Communication Disorders), Social Communication Questionnaire (SCQ) score >15, aged <18 years and medically stable. Exclusion criteria included non-definitive ASD diagnosis, conditions/medications that influence vitamin D metabolism or absorption (including epilepsy), vitamin A supplementation (including cod liver oil) and chronic, non-autistic medical issues.

### Supplements

At baseline, each parent/guardian was provided with eight bottles of drops containing either vitamin D<sub>3</sub> or identical placebo in a double-blind, randomised, age and gender balanced ratio. A physician not involved in data gathering (DC) generated the allocation sequence, while a nutrition researcher enrolled subjects (CPK). Parents/guardians were instructed to administer 20 drops daily for 20 weeks to their child with food (2 000 IU).

Diet, supplement use and particularly exposure to ultraviolet B radiation contribute to vitamin D status. Therefore, we conducted this trial at high latitude (53°N) during winter season when skin vitamin D synthesis is minimal. Further, we advised recruits not to change dietary/supplemental behaviours during the trial. We assessed behaviour relating to vitamin D status with the VIDSun questionnaire at baseline and endpoint.<sup>29</sup> Supplement compliance was assessed with a diary and by returning the bottles of drops at the follow-up visit.

### Study design

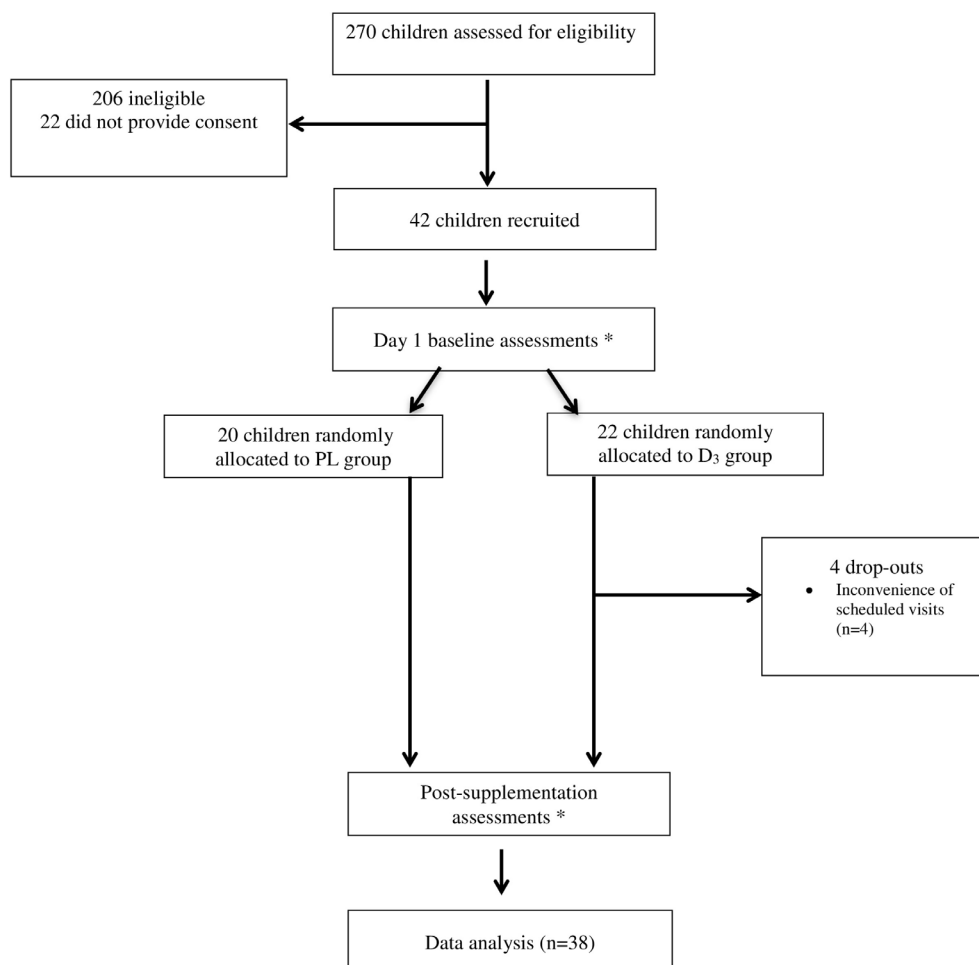
This was a parallel, randomised, double-blind, placebo-controlled trial and involved two clinic visits (figure 1). Recruitment occurred between September and December 2015, with follow-up 20 weeks later. All assessments were conducted at baseline and follow-up. Following completion of day 1 presupplementation assessments, subjects were randomised to vitamin D<sub>3</sub> (D<sub>3</sub>) or placebo (PL) groups using an online randomisation programme.

### Assessments

We used a combination of commonly used parental and clinician subjective autistic measures and biochemical indices.

### Aberrant Behaviour Checklist (ABC)

The ABC, a 58-item informant (eg, parent) rating scale, includes five subscales: irritability, lethargy/social withdrawal,



**Figure 1** Trial design. \*Assessments included subjective autism spectrum disorder measures and blood draw. D<sub>3</sub>, vitamin D<sub>3</sub>; PL, placebo.

stereotypical behaviour, hyperactivity/non-compliance and inappropriate speech. Higher scores indicate greater severity of problem behaviours. ABC is clinically relevant, is sensitive to change, was designed to measure treatment effects and has been used extensively in ASD.<sup>30</sup> Therefore, we selected the stereotypical behaviour subscale of the ABC as our primary endpoint.

#### The Social Responsiveness Scale (SRS)

SRS is another clinically relevant, commonly used, informant rating scale in ASD research.

#### The Developmental Disabilities—Children's Global Assessment Scale (DD-CGAS)

DD-CGAS is a clinician-rated instrument designed to estimate global functioning of the child during the past month across all domains of functioning (self-care, communication, social behaviour and academic functioning).

#### Biochemistry

Venous blood samples were collected at baseline and endpoint and analysed locally for complete blood count, 25(OH)D and C reactive protein (CRP), as well as renal, liver and bone profiles.

We assessed vitamin D status by measuring total serum 25(OH)D. The rationale for measuring 25(OH)D and the methods have been described previously.<sup>31</sup> We dichotomised 25(OH)D levels based the most recent Institute of Medicine (IOM) recommendations where <50 nmol/L indicates VDD and >50 nmol/L indicates vitamin D sufficiency (VDS).<sup>32</sup>

#### Statistics

For this trial, the stereotypical behaviour subscale from ABC served as the primary outcome. We calculated that a sample size of 34 (17 to each group) would provide 81% power at the 0.05 significance level based on an SD of 5.61<sup>26</sup> to detect a treatment difference 5.7.<sup>26</sup> We factored in a drop-out rate of 15% and therefore recruited 42 ASD subjects. All data were normally distributed results and expressed as mean±SD. Paired t-tests were used to assess differences within groups between variables, while unpaired t-tests were used to assess differences between groups. Pearson's coefficient was used for correlation analysis.  $p < 0.05$  was considered statistically significant. Statistical analysis was performed using a software package (SPSS, version 18).

#### RESULTS

We screened 270 children for eligibility. Of these, 64 children were eligible, but only 42 children provided consent, were recruited and completed baseline assessments (figure 1).

#### Baseline findings

All 42 recruited children completed all baseline measures (38 male, mean age 7.1 years; mean body mass index (BMI) 17.6 kg/m<sup>2</sup>). Mean 25(OH)D was 54.2 nmol/L (range: 15–101 nmol/L). According to the IOM guidelines, 17 children were VDD (40%), while 25 were VDS. There were no significant differences in demographics (age and BMI) or serum biomarkers between the VDD and VDS groups.

Using balanced randomisation, children assigned to placebo were well matched to those assigned to vitamin D<sub>3</sub>. However, there were trends toward higher lethargy scores in the D<sub>3</sub> group but lower scores for inappropriate speech and social behaviour.

**Table 1** Baseline demographics

	Placebo	Vitamin D	p Value*
n	20	18	
Age (years)	6.9±3.8	7.9±23.4	0.41
Male, n (%)	18 (90)	15 (83)	–
BMI (kg/m <sup>2</sup> )	17.7±3.9	18.1±2.9	0.69
SCQ	20.2±3.8	19.6±3.1	0.76
ABC			
Irritability	14.1±10.8	14.8±10.8	0.81
Lethargy	10.2±6.3	13.4±7.5	0.08
Stereotypy	7.8±5.2	6.7±4.4	0.5
Hyperactivity	18.6±11.9	19.1±7.9	0.86
Inappropriate speech	5.4±3.8	4.2±2.2	0.3
Total	55.9±32.8	58.3±21.5	0.78
SRS			
Total	150±25.2	149.1±27.2	0.89
DD-CGAS			
Self-care	47±19.1	39.1±20.92	0.24
Communication	46.5±17.3	40.9±18	0.34
Social behaviour	50.5±15.7	42.6±16.4	0.15
School/academic	46.5±17.6	43.2±22.9	0.63
Biochemistry			
CRP (mg/L)	2.7±5.7	1.1±0.4	0.27
Ca <sup>2+</sup> (mmol/L)	2.3±0.06	2.33±0.07	0.36
25(OH)D (nmol/L)	51.7±19.8	58.4±17.9	0.3

\*p Values were derived using two-tailed, independent-samples t-tests. 25(OH)D, 25-hydroxyvitamin D; ABC, Aberrant Behaviour Checklist; BMI, body mass index; Ca<sup>2+</sup>, albumin-corrected calcium; CRP, C reactive protein; DD-CGAS, Developmental Disabilities—Children's Global Assessment Scale; SRS, Social Responsiveness Scale.

#### Follow-up results

Of the 42 recruits, four children dropped out due to inconvenience of scheduled visits (figure 1) (all from the vitamin D<sub>3</sub> group). Therefore, 38 children completed the trial (table 1).

Compliance with trial supplements was high in both groups (>95%) as assessed with a diary and by examining the bottles of drops at follow-up. No adverse effects were reported.

Table 2 displays a comparison of changes in means after 20 weeks. As expected, after 20 weeks of vitamin D<sub>3</sub> supplementation, there was a significant increase in 25(OH)D compared with PL ( $p = 0.0016$ ), but no change in VIDSun score in either group. No effect was observed on the primary endpoint (stereotypical behaviour subscale of the ABC). A significant improvement was observed in self-care score on DD-CGAS in D<sub>3</sub> versus PL ( $p = 0.02$ ). A non-significant decrease in inappropriate speech was observed in PL versus D<sub>3</sub> ( $p = 0.08$ ). No other significant between group differences were observed for the behavioural measurements.

25(OH)D decreased in a single child in the vitamin D supplementation arm (−9 nmol/L) and did not change in a second child. Furthermore, 25(OH)D did not change in two children and actually increased in nine children in the PL group (+1 to +45 nmol/L). Therefore, we analysed the results based on 25(OH)D response (table 3). There was a greater improvement in total SRS score in those with increased 25(OH)D. In contrast, there was a greater improvement in total ABC and ABC subscales in those with decreased 25(OH)D. The three children who had no change in 25(OH)D levels also displayed improvements on ABC, SRS and DD-CGAS.

## Original article

**Table 2** Subjective and biochemical results before and after the trial

n	Placebo			Vitamin D			p Value*
	Pre	Post	Δ	Pre	Post	Δ	
<b>ABC</b>							
Irritability (0–45)	14.1	9	–5.1	14.8	12.3	–3.5	0.45
Lethargy (0–48)	10.2	5.6	–4.6	13.4	10.2	–3.2	0.88
Stereotypical behaviour (0–21)	7.8	4.8	–3	6.7	5.9	–0.8	0.28
Hyperactivity (0–48)	18.6	13.3	–5.3	19.2	15.7	–3.5	0.63
Inappropriate speech (0–12)	5.4	2.9	–2.5	4.2	4.1	–0.1	0.08
Total	55.9	35.5	–20.4	58.3	48.2	–10.1	0.2
<b>SRS</b>							
Total (0–260)	150.3	137.6	–12.7	149.1	124.8	–24.6	0.48
<b>DD-CGAS</b>							
Self-care (0–100)	47	54.5	7.5	39.1	59.4	20.3	0.02
Communication (0–100)	46.5	57.5	11	40.8	52.6	11.8	0.6
Social behaviour (0–100)	50.5	56.3	5.8	40.6	55.6	5	0.38
School/academic (0–100)	46.5	59.5	13	43.2	51.2	8	0.24
<b>Biochemistry</b>							
CRP (mg/L)	2.7	1.5	–1.2	1.1	1.3	0.2	0.4
Neutrophil:lymphocyte	1.1	1.2	0.1	1	1.3	0.3	0.58
Ca <sup>2+</sup> (mmol/L)	2.3	2.33	0.03	2.3	2.3	0	0.26
25(OH)D (nmol/L)	51.7	50.6	–1.1	58.5	86.1	27.6	0.0016

\*p Values derived from unpaired t-tests.

25(OH)D, 25-hydroxyvitamin D; ABC, Aberrant Behaviour Checklist; Ca<sup>2+</sup>, albumin-corrected calcium; CRP, C reactive protein; DD-CGAS, Developmental Disabilities—Children's Global Assessment Scale; SRS, Social Responsiveness Scale.

Because it has been hypothesised that vitamin D supplementation may be most beneficial regarding ASD in early life,<sup>26</sup>

**Table 3** Per cent change in parameters according to 25(OH)D response

	Decreased 25(OH)D	Unchanged 25(OH)D	Increased 25(OH)D
n	10	3	24
PL allocation	9	2	9
D <sub>3</sub> allocation	1	1	15
<b>ABC</b>			
Irritability (0–45)	–38	2	–4
Lethargy (0–48)	–56	–5	–4
Stereotypical behaviour (0–21)	–59	–2	–2
Hyperactivity (0–48)	–42	–4	–3.5
Inappropriate speech (0–12)	–53	0	–1
Total (0–174)	–49	–9	–13
<b>SRS</b>			
Total	–14	–1	–23
<b>DD-CGAS</b>			
Self-care (0–100)	12	17	14
Communication (0–100)	25	17	10
Social behaviour (0–100)	20.7	–18	10
School/academic (0–100)	27	7	11
<b>Biochemistry</b>			
CRP (mg/L)	10	–9	0.1
Neutrophil:lymphocyte	0.2	–1	0.1
Ca <sup>2+</sup> (mmol/L)	2	–0.01	0.1
25(OH)D (nmol/L)	–37	0	25

25(OH)D, 25-hydroxyvitamin D; ABC, Aberrant Behaviour Checklist; Ca<sup>2+</sup>, albumin-corrected calcium; CRP, C reactive protein; DD-CGAS, Developmental Disabilities—Children's Global Assessment Scale; PL, placebo; SRS, Social Responsiveness Scale.

<sup>27</sup> we analysed results based on age. Although irritability improved significantly more in those <6 years compared with those >6 years (p=0.04), there were no other significant differences, including stereotypical behaviour (primary endpoint).

Table 4 presents the correlation coefficients between ABC, SRS and DD-CGAS parameters versus 25(OH)D at both baseline and follow-up. We observed only a single significant correlation

**Table 4** Correlations between ASD parameters and 25(OH)D

	Baseline	Follow-up
<b>ABC</b>		
Irritability	0.29	0.27
Lethargy	0.12	0.04
Stereotypical behaviour	0.18	0.14
Hyperactivity	0.36*	0.19
Inappropriate speech	0.17	0.14
Total	0.3	0.2
<b>SRS</b>		
Total	0.2	0.04
<b>DD-CGAS</b>		
Self-care (0–100)	0.006	0.139
Communication	–0.28	–0.06
Social behaviour	–0.003	0.12
School/academic	–0.16	0.06

\*Significant (p=0.018).

25(OH)D, 25-hydroxyvitamin D; ABC, Aberrant Behaviour Checklist; DD-CGAS, Developmental Disabilities—Children's Global Assessment Scale; SRS, Social Responsiveness Scale.

with baseline 25(OH)D and no significant relationships with follow-up 25(OH)D.

## DISCUSSION

We present one of the first randomised, double-blind, placebo-controlled trials of vitamin D in ASD. We investigated the effect of vitamin D<sub>3</sub> supplementation (2000 IU/day) for 20 weeks among a small sample of urban, mostly Caucasian children with clinician-diagnosed ASD at high latitude over winter season. In contrast to most<sup>11 26 27</sup> but not all previous reports,<sup>21</sup> there was no difference between VDD and VDS groups regarding ASD parameters at baseline. There was a significant increase in vitamin D status in the D<sub>3</sub> group. However, there was no effect on the prespecified primary endpoint: stereotypical behaviour subscale from ABC.

A significant improvement was observed in a single, exploratory outcome (self-care score) with vitamin D supplementation. A non-significant improvement was observed in another single, exploratory outcome (decreased inappropriate speech) in the PL group. These contradictory observations are likely stochastic or statistical artefact. There were no other observed differences between groups.

In the vitamin D arm, 25(OH)D decreased in a single child and did not change in a second child. Although reported compliance with the supplements was high, this could nevertheless be explained by non-compliance (we did not objectively measure compliance). Other explanations include measurement error or absorption issues. We cannot rule out an absorption/metabolic issue with vitamin D which is supported by a recent small study where 25(OH)D was significantly lower in 42 ASD children compared with 40 typically developing children ( $p < 0.001$ ) despite similar sun exposure and oral intake.<sup>33</sup> Furthermore, despite conducting this trial at high latitude over the winter season and the lack of change in VIDSun scores, 25(OH)D remained constant in two children and actually increased in nine children in the PL group (+1 to +45 nmol/L). This could possibly reflect commencement of non-trial vitamin D supplementation or skin photosynthesis of vitamin D, since follow-up occurred in mid-late spring. Based on these observations, we further analysed results based on 25(OH)D response, which revealed inconsistent results. We also conducted regression analysis, which revealed only a single significant relationship (out of 22), again suggesting a stochastic or statistical artefact. Collectively, our results suggest that there was no benefit of vitamin D<sub>3</sub> supplementation in ASD compared with placebo. Furthermore, improvements in the children whose 25(OH)D level either decreased or did not change suggest a significant placebo effect. The placebo effects pose significant challenges in ASD regarding reliable detection of a treatment response. A recent study simulated a clinical trial with baseline and endpoint assessments but no treatment, yet caregivers reported ABC and SRS improvements.<sup>34</sup>

In addition to multiple reports of inadequate vitamin D intake<sup>8–11</sup> and/or vitamin D status<sup>11–21</sup> in ASD, detailed *in vitro* and *in silico* work has demonstrated that relevant genes (eg, THP2 gene) and brain hormones (serotonin, oxytocin and vasopressin) are directly regulated by calcitriol, also known as 1,25-dihydroxyvitamin D<sub>3</sub> (1,25D),<sup>23 35 36</sup> implying that VDD may have a role in ASD. Furthermore, an early report of vitamin D supplementation providing protective and restorative effects on ASD features in rat pups<sup>24</sup> increased interest in the therapeutic potential of vitamin D for ASD. This was followed by a case study reporting improvement of core ASD symptoms following 150 000 IU/month for 2 months+400 IU/day in a 32 month-old

Chinese boy with VDD and ASD.<sup>25</sup> Subsequently, two open-label studies were published. An Egyptian study reported that 67 of 83 VDD children with ASD (81%) had improved ABC and childhood autism rating scale following vitamin D<sub>3</sub> supplementation (300 IU/day/kg of body weight) for 12 weeks.<sup>26</sup> Another open-label study from China assessed 37 VDD children before and after monthly intramuscular vitamin D<sub>3</sub> (150 000 IU) and daily oral vitamin D<sub>3</sub> (400 IU). Total ABC and SRS scores were reduced significantly after 12 weeks.<sup>27</sup> Additionally, a very recent, double-blind, randomised, placebo-controlled trial consisted of 109 ASD children (3–10 years) randomised to daily vitamin D (300 IU/kg of body weight but  $\leq 5000$  IU) or placebo for 4 months. Vitamin D supplementation significantly improved ABC, SRS, childhood autism rating scale and Autism Treatment Evaluation Checklist scores.<sup>28</sup> Although there were many similarities between our trial and this other trial, there are several notable differences. Our cohort were older (mean age 7.4 vs 5.4 years), had lower ABC scores (and perhaps milder ASD) and had lower baseline 25(OH)D (54.2 versus 66 nmol/L). Furthermore, the change in 25(OH)D was lower in our trial (+25 versus +49 nmol/L) resulting in a much lower 25(OH)D level at follow-up (83.8 versus 114.8 nmol/L).

## Trial strengths

We used the gold standard study design and assessed a combination of widely used subjective assessments and biochemical markers. The PL and vitamin D groups were well matched at baseline with no significant differences in demographics, including 25(OH)D.

Vitamin D trials can be influenced by a number of factors, including fluctuations in sun exposure, variable quality of vitamin D assays, compliance with the intervention and provision of inadequate vitamin D supplementation doses. To overcome these factors, we conducted this trial over the winter season at high latitude when vitamin D photosynthesis is minimal. Although we purposely did not restrict non-protocol dietary or supplemental vitamin D intake, we did ask that such behaviours were not altered during the trial. Vitamin D behaviours were similar in both groups throughout the trial. We used the current gold standard assay for total 25(OH)D. Reported supplement compliance was high. We used a moderate-to-high dose of vitamin D<sub>3</sub> (2000 IU/day). Finally, comorbidities and medication use were rare in both groups. Furthermore, there was no change in non-trial medication/therapy/supplementation.

## Trial limitations

Limitations of our study include the relatively small number of subjects. We used fixed vitamin D dosing, but it may be more appropriate to titrate vitamin D dosing based on baseline 25(OH)D levels and/or body size.<sup>24 26 28</sup>

We did not selectively recruit VDD children. However, any detrimental effect of low 25(OH)D may be most apparent when comparing VDD with VDS. In this trial, mean baseline 25(OH)D was 54.2 nmol/L (range: 15–101 nmol/L). Only 17 children were VDD (40%). However, previous open-label studies<sup>26 27</sup> and a randomised controlled trial also included subjects who were not VDD<sup>28</sup> which all demonstrated benefit of vitamin D supplementation.

Although 25(OH)D is considered the best circulating biomarker of vitamin D status, there are many important metabolites and intermediates in the vitamin D metabolic pathway. For example, paternal<sup>37</sup> and individual<sup>38 39</sup> vitamin D receptor (VDR) polymorphisms have been associated with increased ASD

## Original article

risk. We did not measure levels of vitamin D-binding protein, VDR or calcitriol (1,25D) which may have provided interesting insight here.

Stereotypical behaviour was selected as the primary outcome measure based on a single, open-label study reported prior to the design of this study.<sup>26</sup> To our knowledge, there are no mechanisms by which vitamin D could specifically improve stereotypical behaviour. However, another recent trial also reported a significant improvement in stereotypical behaviour in ASD after vitamin D supplementation.<sup>28</sup>

It is possible that we studied children who were too old to gain neurodevelopmental benefit from vitamin D supplementation. Furthermore, vitamin D supplementation has been suggested to have primary prevention potential regarding ASD.<sup>38–44</sup> Furthermore, vitamin D supplementation has been suggested to have primary prevention potential regarding ASD.<sup>38–43</sup> Although we analysed results based on age (ie, <6 vs 6 years), vitamin D treatment effects have been reported to be more pronounced in younger children (<3 years) with ASD<sup>27</sup> leading to the suggestion that vitamin D supplementation should start in early infancy or during gestation.<sup>26–27–39–44</sup> In this context, it is possible that there is a window of opportunity for adequate 25(OH)D to provide neuroprotection and that we included older children in whom neuronal networks are established and therefore less likely to benefit from supplementation (mean age in vitamin D group 7.9 years).

Finally, it is possible that a higher dose of vitamin D would produce a greater change in both 25(OH)D and ASD behaviour. Some reports have demonstrated selective success with vitamin D supplementation when 25(OH)D exceeds 100 nmol/L.<sup>26–28</sup> In this study, only five children (13%) obtained a 25(OH)D >100 nmol/L.

## CONCLUSIONS

Our results do not support supplementary vitamin D for children with ASD. Considering preliminary interventional studies of 8–16 weeks<sup>26–28</sup> as well as the extent of vitamin D deficiency in ASD and the low cost and high benefit to risk ratio of vitamin D supplementation, larger and preferably longer trials, perhaps focusing on primary prevention and/or younger children as well as relevant mechanisms and optimal dose/duration of supplementation/25(OH)D levels with ASD, are warranted.

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**Contributors** CPK conceptualised and designed the study and data collection instruments, coordinated and supervised data collection, carried out the initial analyses, drafted the initial manuscript and approved the final manuscript as submitted. CP reviewed, coordinated and supervised data collection; revised the manuscript and approved the final manuscript as submitted. LG designed the data collection instruments and approved the final manuscript as submitted. DC conceptualised and designed the study, carried out the initial analyses and approved the final manuscript as submitted.

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## Lack of effect of vitamin D<sub>3</sub> supplementation in autism: a 20-week, placebo-controlled RCT

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