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Long-term Vitamin D3 Supplementation in Japanese Children with Autism Spectrum Disorder: A Pilot Study

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Abstract

Background: Serum 25-hydroxyvitamin D deficiency is frequently observed in children with autism spectrum disorder. Although vitamin D supplementation has shown efficacy in alleviating symptoms in populations outside Japan, there is limited evidence regarding its long-term effects in Japanese children with autism spectrum disorder. This study aimed to evaluate the effects of vitamin D supplementation on serum 25-hydroxyvitamin D concentrations and core clinical symptoms in this population.

Methods: Three-year-old children diagnosed with autism spectrum disorder and presenting insufficient serum 25-hydroxyvitamin D concentrations received vitamin D supplementation at 5.0 μg/day. Autism symptoms were assessed using the Childhood Autism Rating Scale to evaluate autism severity and the Short Sensory Profile to characterize sensory processing patterns.

Results: Vitamin D supplementation increased serum 25-hydroxyvitamin D concentrations, which decreased after a four-month washout. Reintroduction of supplementation restored serum 25-hydroxyvitamin D concentrations. Childhood Autism Rating Scale scores showed a decrease in children with mild-to-moderate autism spectrum disorder following long-term supplementation, whereas no reduction was observed in children with severe autism. Short Sensory Profile scores showed a trend toward improvement.

Conclusion: These findings suggest that long-term vitamin D supplementation may contribute to the improvement of core symptoms and sensory processing characteristics in children with mild-to-moderate autism spectrum disorder. However, its therapeutic benefit appeared limited in children with severe autism.

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Introduction

VitaminD is a fat-soluble vitamin in two forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). It is obtained through dietary intake and synthesized endogenously in the skin in response to ultraviolet (UV) light exposure. Cutaneous synthesis is self-regulated, and excessive sun exposure does not result in overproduction of vitamin D [1]. Vitamin D deficiency and insufficiency represent a global health concern [4], as inadequate vitamin D status can impair calcium and phosphorus absorption in the small intestine and kidneys, increasing the risk of rickets in children and osteomalacia in adults. However, excessive intake can cause hypercalcemia, renal impairment, and soft tissue calcification [1].

Serum 25-hydroxyvitamin D (25[OH]D) concentration reflects total vitamin D status, integrating contributions from both UV-induced synthesis and dietary sources [2], and is therefore recognized as a reliable biomarker for assessing vitamin D sufficiency.

In 2022, the National Health and Nutrition Survey reported that the mean daily intake of vitamin D among the Japanese population (total, male/female, per capita) was 6.2 μg, which falls below the recommended 8.5 μg [3]. Risk factors for vitamin D deficiency in Japanese children include exclusive breastfeeding, maternal vitamin D deficiency, inadequate dietary intake, dietary restrictions related to food allergies, and insufficient outdoor activity [5].

A Japanese study revealed that the vitamin D concentrations in breast milk were lower in 2016–2017 than in 1989 [6]. Infants who are exclusively breastfed have a higher incidence of vitamin D deficiency than those who are formula-fed or receive a combination of breast milk and formula [7]. In a separate study of 50 infants aged 0-5 months, 17 (34.0%) exhibited vitamin D deficiency (<12 ng/mL), while 9 (18.0%) demonstrated vitamin D insufficiency (12-20 ng/mL) [8].

Autism spectrum disorder (ASD) arises from a complex combination of genetic and environmental factors [9]. The core characteristics of ASD include persistent deficits in social communication and interpersonal interactions alongside restricted and repetitive patterns of behavior, interests, or activities [10]. Numerous studies have explored the prevalence and etiology of ASD [11-14]; the exact causes of the disorder remain undetermined, with both environmental and genetic factors playing contributory roles.

The potential association between ASD and vitamin D deficiency was initially proposed by Cannell in 2008, who hypothesized that an increase in ASD prevalence over the preceding two decades coincided with increased medical advisories against sun exposure, resulting in reduced vitamin D levels [15]. Since then, numerous studies in Europe

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and North America have investigated this association. According to a meta-analysis, children with autism spectrum disorder (ASD) tend to have higher levels of 25(OH)D deficiency or insufficiency compared to typically developing children [16]. Furthermore, randomized controlled trials have demonstrated that vitamin D supplementation significantly improves core symptoms of ASD in children [17]. However, the effects of vitamin D supplementation on ASD symptoms in Japanese children remain poorly understood.

The study aimed to monitor serum 25(OH)D concentrations over time in Japanese children diagnosed with ASD and to evaluate the impact of sustained vitamin D supplementation on the severity of core symptoms.

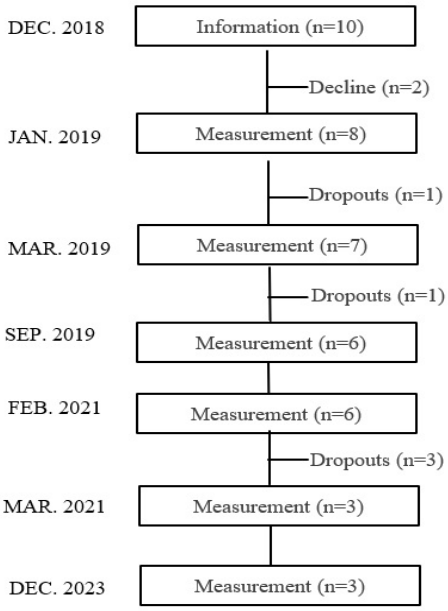
Material and Methods

Subjects and setting

This study included three-year-old children (boys and girls) who had been diagnosed with ASD by a pediatric psychiatrist. Written informed consent was obtained from the parents of all participating children. The study protocol was approved by the Ethics Committee of Bukkyo University (project registration number: 7, 2018). The researchers were present at the child welfare institution (Mukunoki-en, Kyoto, Japan) where the study was conducted to assure the proper management of safety and confidentiality in the study. The manager of the institution invited parents to participate in the study, and all the children whose participation was requested from January 2019 were enrolled.

It was designed to pharmacologically evaluate the effect of a nine-month course of vitamin D supplementation in Japanese children with ASD, including a four-month washout period to allow for assessment of 25(OH)D disposition. Participant dropout was substantial, with many participants failing to complete the study primarily due to disruptions caused by the coronavirus disease 2019 (COVID-19) pandemic. Additionally, to prevent the risk of infection transmission, certain measurements could be performed at the pre-designated time point (Table 1).

Table 1: Impact of the COVID-19 pandemic on the number of study.



All participants took an oral vitamin D supplement (Baby D® 200: vitamin D3 oil 5.0 μg/day purchased from Morishita Jintan Co., Ltd., Osaka Prefecture).

This dose was selected based on a review of existing literature and consideration of the tolerable upper intake level (UL) specified in the "Dietary Reference Intakes for Japanese (2020)." In a previous study by our research group, it was reported that serum 25(OH) D concentrations in Japanese children with ASD were significantly lower than those in typically developing children [18]. Since vitamin D can be obtained through both dietary intake and skin synthesis, a dosage of 5.0 μg/day, which is less than one-third of the UL for each age group, was deemed appropriate.

Serum25(OH)D Assay

Blood was collected by venipuncture and serum 25(OH)D concentrations were measured by Kyoto Microbio Laboratory, (Kyoto, Japan).

The concentrations were interpreted according to the "Guidelines for Determining Vitamin D Insufficiency/Deficiency" [19], issued by the Japanese Society of Bone Metabolism, the Japan Endocrine Society, and affiliated organizations: ≥30 ng/mL was classified as vitamin D sufficient; 20–30 ng/mL as vitamin D insufficient; and <20 ng/mL as vitamin D deficient.

Adaptive function test

The assessment of autism spectrum disorder (ASD) was conducted by occupational therapists with extensive experience in evaluating children with ASD, using developmental assessments that included the Autism Rating Scale (CARS) and the Short Sensory Profile (SSP).

The CARS is used to diagnose ASD and determine its severity by evaluating 15 behavioral items. These items include: (1) Social Interaction, (2) Imitation, (3) Emotional Response, (4) Body Use, (5) Object Use, (6) Adaptation to Change; (7) Visual Response; (8) Auditory Response; (9) Taste, Smell, tactile responses and use; (10) fear and anxiety; (11) verbal communication; (12) nonverbal communication; (13) activity level; (14) level and balance of intellectual functioning; and (15) overall impression and communication ability of the child. A semi-structured interview was conducted with the parent or guardian, and each item was rated on a 7-point scale: 1 point (within normal range), 2 points (mild abnormality), 3 points (moderate abnormality), and 4 points (severe abnormality). For behaviors falling between two defined levels, a 0.5-point increment was applied. The Japanese version of CARS was standardized by Kurita et al. [20]. Based on the total CARS score, the severity of autism spectrum disorder (ASD) was classified as follows: 29.5 points or below, no ASD; 30–36.5 points, mild to moderate ASD; 37.0 points or above, severe ASD.

SSP was developed by Dunn in the United States for children aged 3-10 years. It is used to assess sensory processing characteristics and consists of 38 items categorized into seven categories: (1) tactile sensitivity; (2) taste and smell sensitivity; (3) motor sensitivity; (4) slowness of response and sensory exploration behavior; (5) auditory filtering; (6) low energy/physical weakness; and (7) visual/auditory sensitivity. A semi-structured interview was conducted, and caregivers rated each item on a 5-point Likert scale: 1 (none), 2 (rarely), 3 (occasionally), 4 (often), and 5 (always). Matsuda et al. [22] classified the total SSP score based on the mean and

standard deviation (SD) of a standardized sample as follows: 80–190 points, Very high (suggesting problematic sensory processing; average + 2SD or higher; 97.7th percentile or higher); 58–79 points, High (suggesting questionable sensory processing; between average + 1SD and average + 2SD; 84.1st to 97.7th percentile); and 38–57 points: average (typical sensory processing; < average + 1 SD; < 84.1st percentile) [22]. Very high scores in any category may indicate significant difficulties in sensory stimulus processing.

Results

Change in serum 25(OH)D

Children diagnosed with ASD had vitamin D insufficiency and deficiency. Vitamin D supplementation increased serum 25(OH)D concentrations to levels comparable to those previously observed in typically developing children [19]; however, concentrations decreased during the subsequent washout period. Upon reinitiating vitamin D supplementation, serum 25(OH)D concentrations (Figure 1).

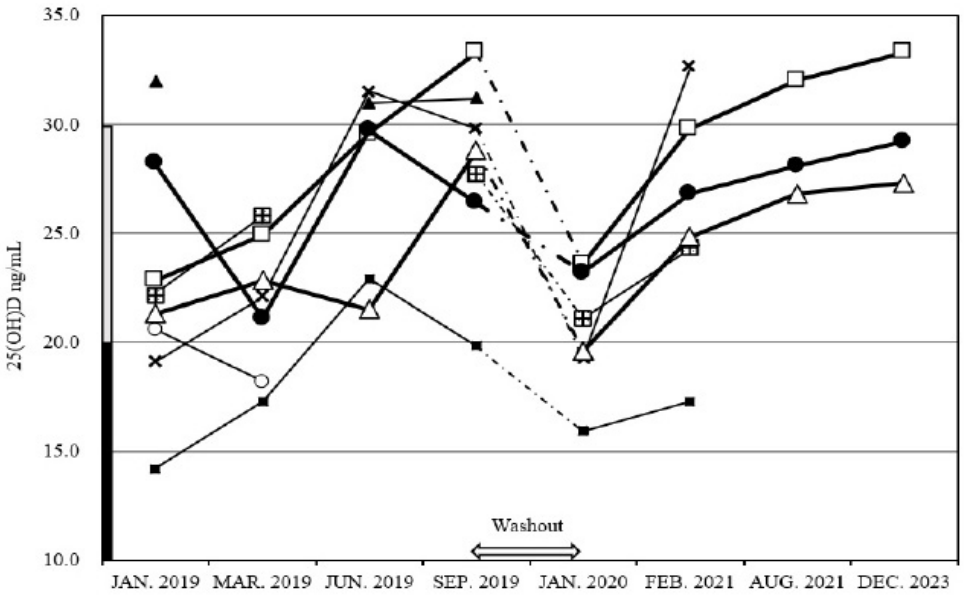


Figure 1: Changes in serum 25(OH)D concentrations over four years.
After nine-months of vitamin D3 supplementation, a four-month washout period was implemented, followed by the resumption of supplementation. Black bars represent indicates a deficiency, and grey bars represent indicates insufficient.

Adaptive function

CARS scores revealed a trend toward reduction of mild-to-moderate ASD following long-term vitamin D supplementation. After approximately four years of continuous supplementation, CARS scores in these children decreased from 28 to 22 and from 36 to 25, respectively. However, children classified as having severe ASD showed no improvement; their CARS scores increased from 41 before supplementation to 44 afterward (Figure 2).

SSP scores also showed a trend toward improvement in children with mild to moderate ASD and those with severe ASD following vitamin D supplementation. SSP scores decreased from 82 to 40 and from 116 to 60, respectively. However, in children classified as having severe ASD, SSP scores were 99 to 83, showing a more gradual decrease (Figure 3).

Discussion

Wang et al. reported that vitamin D deficiency in children with ASD has been reported to be independent of race or ethnicity [23]. Consistent with our study, children with ASD exhibited serum vitamin D levels categorized as insufficient or deficient in comparison to typically developing children.

We hypothesized that elevated serum 25(OH)D concentrations achieved through supplementation could be sustained over time. However, the washout period resulted in a decrease in serum 25(OH) D concentrations in children with ASD, necessitating the resumption of supplementation to restore these levels. Following the washout phase, vitamin D supplementation was continued for approximately three years. No excessive or uncontrolled elevation in serum 25(OH) D concentrations was observed during this period. Notably, serum vitamin D levels improved in one child from deficiency to sufficiency, while two children remained in the deficient range. These results suggest that long-term vitamin D supplementation may effectively improve and maintain serum 25(OH)D concentrations in children with ASD presenting with vitamin D deficiency or insufficiency.

Children with mild-to-moderate ASD exhibited decreased CARS scores following vitamin D supplementation, indicating a reduction in ASD severity. This decrease was not observed in children with severe ASD. Children with mild-to-moderate ASD exhibited greater improvements in serum 25(OH)D concentrations following supplementation compared to those with severe ASD. This may therefore contribute to sustained reductions in CAR scores. A previous case-control cross-sectional study by Saad et al., involving 122 Egyptian children with ASD, reported significantly lower serum 25(OH)D levels in children with severe ASD compared to those with mild-to-moderate ASD. Additionally, serum 25(OH)D levels demonstrated a significant negative correlation with CARS scores [17]. Collectively, these results suggest that maintaining adequate

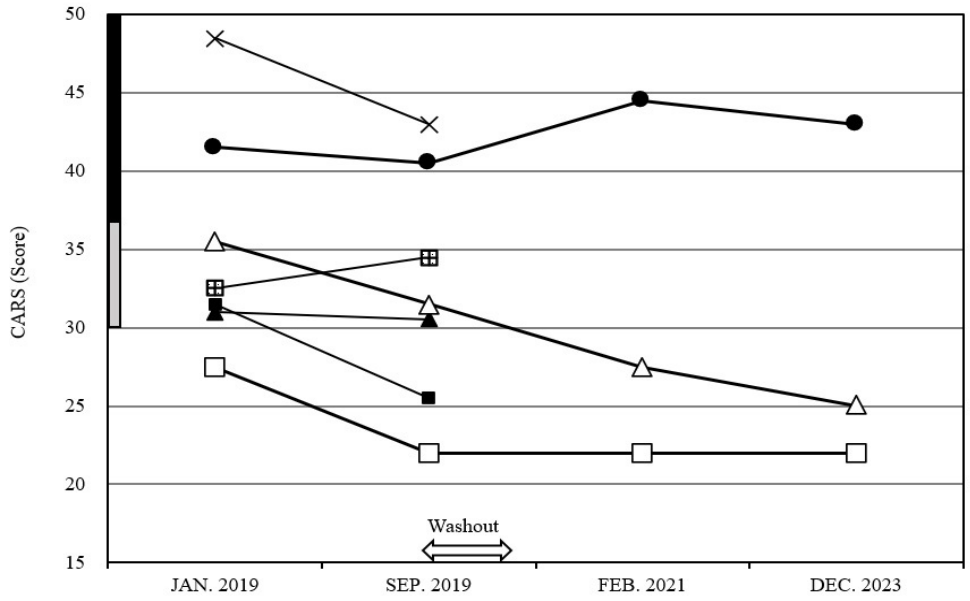


Figure 2: Changes in CARS scores over four years.
After nine months of vitamin D3 supplementation, a four-month washout period was implemented, followed by the resumption of supplementation. Black bars represent severe ASD, and grey bars represent mild to moderate ASD.

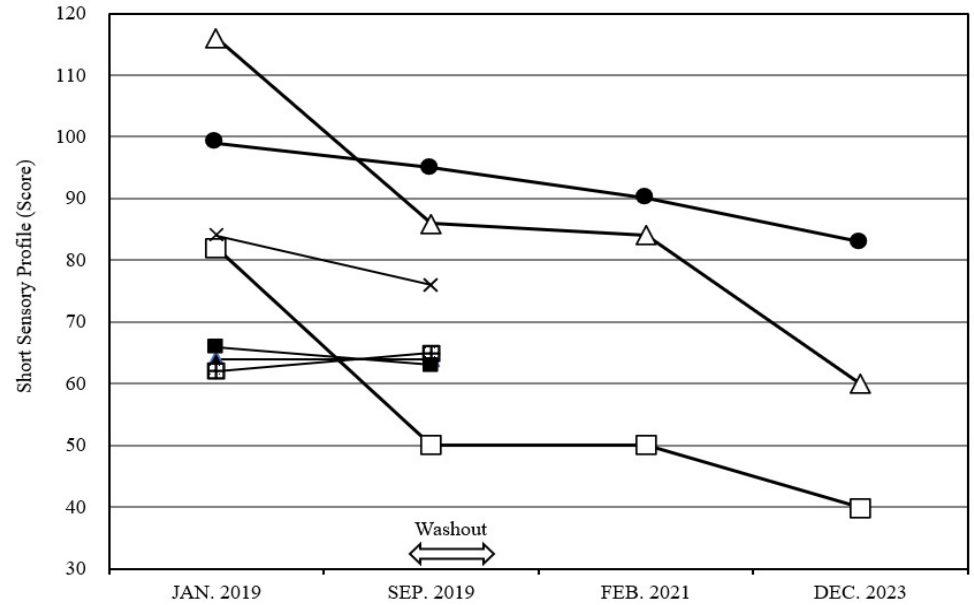


Figure 3: Changes in SSP scores over four years.
After nine months of vitamin D3 supplementation, a four-month washout period was implemented, followed by the resumption of supplementation.

vitamin D levels in children with mild-to-moderate ASD may contribute to a reduction in ASD severity, as evidenced by lower CARS scores.

A negative correlation between serum 25(OH)D level maintenance and CARS scores has been reported previously [17]. In this study, particular attention was given to the rate of increase in 25(OH)D concentrations following the washout period as a potential factor underlying the lack of CARS score improvement in children with severe ASD. After the washout period, serum 25(OH)D concentrations were increased by 128.1% and 146.0% in children with mild and moderate ASD, respectively: however, the increase was only 103.5% in the child with severe ASD. These findings suggest a blunted response in serum 25(OH)D concentrations and a lack of improvement in CARS scores among children with severe ASD, despite continued vitamin D supplementation.

A PubMed search revealed no prior studies examining the relationship between serum 25(OH)D concentration maintenance

and SSP scores, nor any investigations into the association between nutrients, including vitamin D, and SSP scores. However, the results of this study suggest that sustained serum 25(OH)D concentrations may contribute to improvements in abnormal sensory responses among children with ASD.

Although the number of participants was limited, this study demonstrated differential improvements in sensory processing characteristics between children with mild-to-moderate and severe ASD. We hypothesize that 25(OH)D disposition may differ according to ASD severity. A study by Shom et al. conducted in India reported that deficiencies in both 25(OH)D and vitamin D-binding protein (DBP) were associated with increased ASD severity [25]. Similarly, Saechua et al. reported that genetic polymorphisms within the vitamin D metabolic pathway in Thai children were associated with increased susceptibility to and severity of ASD [26].

Vitamin D-DBP regulates the storage and transport of vitamin D in the circulation but also plays a critical role in controlling serum 25(OH)D levels [27]. DBP deficiency and genetic polymorphisms affecting the vitamin D metabolic pathways may account for the limited improvements in CARS and SSP scores observed in children with severe ASD compared to those with mild-to-moderate ASD in this study.

This study has several limitations. It was a preliminary investigation including a small sample of Japanese children with ASD, and no targeted analyses were conducted. The study was initiated before the coronavirus disease 2019 (COVID-19) pandemic, during which a substantial number of participants withdrew. Additionally, infection control measures impeded data collection at predesignated time points, complicating direct comparisons. Although novel insights were obtained through long-term observations, the small sample size precluded statistical analysis. Future studies with larger cohorts, assessments of DBP deficiency and relevant genetic polymorphisms, and robust statistical analyses are warranted to validate and extend these findings.

Conclusion

Despite the number of participants in this study being very limited, the findings indicate that children with mild-to-moderate ASD exhibited reductions in CARS and SSP scores following vitamin D supplementation. The sustained maintenance of serum 25(OH)D concentrations through long-term supplementation may contribute to improvements in ASD severity and sensory processing characteristics, potentially mitigating the progression from mild to moderate to severe ASD. These results suggest that long-term vitamin D supplementation in children with ASD may have both clinical and broader social implications.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

Ms. Mochizuki contributed to the study conception and design, interpretation of data, and drafting of the manuscript.

Dr. Yamada was responsible for data acquisition and critical revision of the manuscript.

Dr. Hasegawa contributed to data interpretation and critically reviewed the manuscript.

Dr. Kida contributed to data interpretation and critically reviewed the manuscript.

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