

**Lay abstract:** Vitamin D is a nutrient believed to be important in brain development and function. Previous research has suggested that there may be a relationship between lower levels of vitamin D and Autism Spectrum Disorder (ASD). Yet it is unknown whether lower vitamin D levels come before an ASD diagnosis or alternatively, are a consequence of ASD. The aim of this study was to evaluate whether vitamin D levels (measured prior to an ASD diagnosis), were related to ASD. We assessed vitamin D blood levels and vitamin D supplementation prior to ASD diagnosis among 3852 children, all below the age of 6 years who were participating in The Applied Research Group for Kids (TARGet Kids!) study. A review of children's medical records identified 41 children with ASD diagnoses. We found that early childhood vitamin D levels were not related to whether a child received a subsequent diagnosis of ASD. Taking vitamin D supplements was also not related to subsequent ASD diagnoses. Overall, the findings suggest that vitamin D levels in early childhood may not be related to ASD diagnosis. Other time periods (such as during pregnancy) may be important to assess whether vitamin D affects ASD.