# Efficacy of Vitamin D Supplementation on Bone Mineral Density and Vitamin D Binding Protein in Children with Autism

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**Abstract:** **Background:** Autism spectrum disorder is a neuro-developmental disorder, which is reported to affect 1 in 59 children. Social communication deficits and restricted or repetitive interests and behaviors characterize it. It is caused by the interaction of both genetic and environmental factors. **Objective:** To assess vitamin D, vitamin d binding protein (VDBP) and bone mineral density in autistic children, compared to typically developing children, also to question the benefit of using oral vitamin D daily supplementation for 4 months on autistic children’s outcome. **Patients and Methods:** The study was conducted on 30 autistic patients recruited from the Autism Disorders Clinic, Medical Research Centre of Excellence, and National Research Centre with age ranging from 3-7 years. Patients were randomly supplemented by vitamin D in a dose of 600 IU/day for 4 months. Thirty healthy children were included as control, with matched age and socioeconomic status to the study group. Exclusion criteria for the patients’ group were known genetic syndromes, static or progressive neurologic conditions, children on dietary restriction, non -ambulatory patients, and patients on drugs that affect vitamin D metabolism. Regarding the exclusion criteria of the control group, they were not on either vitamin D supplementation or drugs that affect vitamin D. **Results:** The results of the study revealed that autistic group of study have vitamin D deficient. However, there was no significance between patients before and after supplementation. On comparing VDBP levels of the patients and controls groups, we discovered statistically highly significant difference in level of it. but, There was significant difference of its level between before and after supplementation in autistic patients. In addition, we revealed that low bone mineral density DEXA of both regions. (Neck femur and lumber) were discovered. While, it was no significant difference between autistic children (before and after) supplementation. **Conclusion:** Wrapping up, it is still premature to conclude that autism and vitamin D are both related to each other. And although this work did not uncover any of the mysteries of autism, yet it highlighted the tight spot of vitamin D deficiency in children in general, and autistic children in particular who are more prone to vitamin D deficiency complications as regard bone metabolism and the higher risk for immobilization. Vitamin D also exhibits poor water solubility and oral bioavailability. Further studies should be implemented on a larger group of autistic children, and longer duration of vitamin D supplementation to reach optimal blood vitamin D levels. We recommend vitamin D supplementation using nanotechnology to overcome associated problems with administration.

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**Introduction**

Autism spectrum disorder (ASD) is a neuro-developmental disorder of increasing prevalence in the modern era. Presently, this condition is reported to affect 1 in 50 children ***(Center for Disease Control and Prevention, 2013)***. It is characterized by social communication deficits and restricted or repetitive interests and behaviors. Children with ASD present along a wide spectrum of clinical severity, from mild social difficulties to severe functional impairment. This condition typically presents in the first 3Description: Description:  years of life, manifested by a failure to gain, or a loss of social communication milestones ***(Rosenzweig et al 2017).***

Children with autism have distinctive eating habits that result from the nature of the disease characteristics ***(Cascio et al., 2012)*** that can limit dietary variety and put children at risk for nutritional deficiencies ***(Mazumdar et al., 2012).***

Low levels of vitamin D in blood among children with ASD have been reported ***(Meguid et al., 2010)***.

Furthermore, Vitamin D during pregnancy has been reported as a risk factor for ASD development in offspring ***(Cannell, 2008; Grant and Soles, 2009)***.

In 2011, the National Institute of Health estimated the normal level for 25 OHD to be (above 30 ng/ml), while the Level between (20-30ng/ml) estimated to be insufficient, and less than 10 ng/ml Is deficient.

Vitamin D is necessary for gastrointestinal absorption of calcium and phosphorus, as well as for reducing the renal excretion of calcium ((Cristina Rusu et al., 2015). It is naturally present in a small number of foods, including fatty fish and liver **(Kinner and Moody, 2010)** and fortified food products such as cow’s milk. In 2008, the American Academy of Pediatrics recommended doubling vitamin D intake benchmarks for infants and children to be400 IU/day **(Wagner and Greer, 2008).**

# Autism

Autism is one of a group of neurodevelopmental disorders known as pervasive developmental disorders (PDD). These disorders are characterized by three core deficits: impaired communication, impaired reciprocal social interaction and restricted, repetitive and stereotyped patterns of behaviors or interests. The presentation of these impairments is variable in range and severity and often changes with the acquisition of other developmental skills. With the development of the fifth Diagnostic and Statistical Manual of mental disorders (DSM-V), the term of Autism Spectrum Disorder replaces the term Pervasive developmental Disorders ***(Opal and Cermak., 2015)***.

History:

In 1943, the American psychiatrist Leo Kanner used the term ''early infantile autism" to describe children who lacked interest in other people. In 1944, an Austrian pediatrician, Hans Asperger, independently described another group of children with similar behaviors, but with milder severity and higher intellectual abilities.

It was until the 1980s that the term pervasive developmental disorders was first used. The definition and diagnosis of these disorders has been broadened over the years to include milder forms of autism. The term autism spectrum disorders (ASDs) is currently used to describe three of the five pervasive developmental disorders listed in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and the international Classification of Diseases, Tenth (ICD-10), Autistic disorder, Asperger disorder and pervasive developmental disorder-not otherwise specified (PDD-NOS) ***(***[***McPartland***](https://www.ncbi.nlm.nih.gov/pubmed/?term=McPartland%20J%5BAuthor%5D&cauthor=true&cauthor_uid=22608634) ***and***[***Volkmar***](https://www.ncbi.nlm.nih.gov/pubmed/?term=Volkmar%20FR%5BAuthor%5D&cauthor=true&cauthor_uid=22608634)***., 2013).***

A diagnosis of autistic disorder is made when there are impairments in communication and reciprocal social interaction with the presence of restricted repetitive and stereotyped patterns of behaviors or interests, prior to the age of 3 years. When autistic symptoms are present with no significant general delay in language and cognitive development, a diagnosis of Asperger disorder is made. A diagnosis of PDD-NOS is given when the triad of symptoms is present but the criteria are not met for a specific PDD (**Dasal T.*, 2014)*.**

Often the label "high-functioning autism" is used interchangeably with Asperger disorder. This is controversial and there is considerable debate as to whether children with Asperger disorder, who have normal language milestones, should be considered to comprise a subgroup distinct from high-functioning children with autism, who have a history of delayed language development ***(McAlonan et al., 2008)***.

The other two PDD's, Rett syndrome and childhood disintegrative disorder, are rare and are associated with significant developmental regression, which makes them more distinct than the other disorders in the PDD group (**Dasal T.*, 2014)*.**

Rett syndrome is a relatively rare neurological disorder found primarily in females with implications in various areas of independent functioning. A diagnosis in its relative infancy, Rett syndrome results in a diverse array of symptoms including developmental deficiencies in communication, gross motor skills, social interactions, and intellectual abilities. Evidenced across various cultural groups, the rarity of occurrence makes Rett syndrome a relatively ignored topic in counseling literature. In light of the removal of Rett syndrome as a mental health diagnosis from the DSM-5 and misconceptions regarding this decision, this paper investigates the uniqueness of Rett syndrome and provides suggestions for care. A case illustration is provided to illustrate some considerations for the counseling professional ***(International Rett Syndrome Foundation, 2014)***.

Although researchers have identified a genetic cause of Rett syndrome, such causes are not linked to inheritance among populations (National Institute of Child Health and Human Development, 2014). Instead, researchers have indicated that in more than 99% of cases of an individual diagnosed with Rett syndrome, the mutations have occurred spontaneously and at random (International Rett Syndrome Foundation, 2014). This means that only a small portion of individuals with Rett syndrome had a relative who was also diagnosed with the disorder. Further complicating the research, a number of individuals with Rett syndrome were found to have a relative with mutations on their Ideas and Research You Can Use: VISTAS 2015 3 MECP2 gene without demonstrating any clinical symptoms of the disorder, earning these individuals the title of asymptomatic carriers ***(Dolce, Ben-Zeev, Naidu, & Kossoff, 2013)***.

# Vitamin D

Physiology of vitamin D:

Vitamin D was first discovered during the industrial revolution, when England was struck by an unprecedented epidemic of rickets. In 1918, Sir Edward Mellanby demonstrated that the disease was caused by a nutritional deficiency and, soon after, rachitic infants were cured with cod liver oil.

Vitamin D is a fat-soluble vitamin. His **two isoforms; vitamin D2 (ergocalciferol) and vitamin D3** (cholecalciferol) are different only in their side chain structure ***(Eyles et al., 2009).*** In 1965, Robert Bruns Woodward was awarded a Nobel Price for having synthesized vitamin D and vitamin B12.

**Its role** has long been known for its regulating body levels of calcium and phosphorus and in mineralization of bones via receptors for Vit D (VDR) present in a wide variety of cells because it acts as a steroid hormone ***(Nair and Maseeh, 2012)******.***

The primary source of vitamin D (VD) is ultraviolet B rays (UVB)-induced conversion of 7 dehydrocholesterol to VD in the skin. Vitamin D undergoes two hydroxylation processes in the body for activation. Calcitriol (1,25-dihydroxyvitamin D3), the active form of VD, has a half-life of about 15 h, while calcidiol (25-hydroxyvitamin D3) has a half-life of about 15 days. The **major target tissues** for 1,25-dihydroxyvitamin D (1,25 OH VD) **are** bone, intestine and kidney ***(Mollazadeh et al., 2014).***

Linking vitamin D and neuropsychiatric disorders has only received attention in the last two decades ***(Eyles et al., 2009)***. The first indirect clue that vitamin D may have some role in the brain and its metabolites were discovered in the cerebrospinal fluid of healthy adults ***(Anjum et al., 2018)***.

Establishing the presence of the vitamin D receptor (VDR) in the central nervous system (CNS) by immunohistochemical studies in the brains of several species provided the first real clue that vitamin D may have a role in brain function ***(Anjum I. et al., 2018)***.

**Vitamin D signaling in the brain**

VDR is first expressed in the developing brain during critical periods of cell proliferation, suggesting that vitamin D signaling may participate in the highly complex ‘orchestration’ of brain development ***(Eyles et al., 2011)***. The receptor for vitamin D (VDR) is widely distributed in the mammalian brain, it is present in both neuronal and non-neuronal cells from both rodent and human brains. **VDR is expressed widely in the adult brain in the areas that are associated with cognitive functioning as** Cortex, cerebellum and limbic system (hippocampus, cingulate gyrus, amygdale) ***(Mollazadeh et al., 2014).*** 1,25-(OH)2D could act in a paracrine/ autocrine fashion in nervous system ***(Kalueff and Tuohimaa, 2007)***.

**Diagnosis of vitamin D deficiency:**

Obtaining a serum 25(OH)D level is the only way to make the diagnosis. Symptoms of muscular weakness, heaviness in the legs, chronic musculoskeletal pain, easy fatigue may be the keys of vitamin D deficiency. Such complaints are common, difficult to treat, and easy to misdiagnose, but they may indicate symptomatic vitamin D deficiency***.*** Diagnosis is confirmed with a 25-hydroxyvitamin D level of less than 20 ng/mL, while the normal level is between 40-70 ng/Ml ***(Bordelon et al., 2009).***

There are many causes of vitamin D deficiency, despite growing attention to this deficiency; there are no established guidelines to help clinicians decide which patients warrant screening laboratory testing. The risk groups include, older people admitted to hospital, patients with hip fracture, dark-skinned children and mothers with oesteoprosis (***Anjum et al., 2018)***.

**Symptoms of vitamin D toxicity**

**vitamin D** are secondary to effects of hypercalcemia when its serum level gradually increases above 150ng/mL, and rare cases it may calcifies internal organs, especially the kidneys, and this for Practitioners who use doses above 2,000 IU per day. Periodic monitoring will also educate the practitioner not only to the safety of supplementation, but to the surprisingly high oral dose required to achieve and maintain adequate serum 25(OH)D levels, especially in the fall and winter ***(Cannell et al., 2008)***.

The only absolute contraindication to vitamin D supplementation is vitamin D toxicity or allergy to vitamin D, although no reports in the literature were found of acute allergic reactions to vitamin D supplements ***(Cannell et al., 2008)***.

Although the liver initially metabolizes vitamin D, liver disease is not a contraindication to treatment of deficiency. The liver conserves the ability to hydroxylate vitamin D despite advanced liver disease. ***(Handunnetthi et al., 2010)***.

**Autism spectrum disorder**

Vitamin D deficiency either during pregnancy or early childhood has recently been proposed as a possible environmental risk factor for ASD ***(Grant and Soles, 2009)***.

**Humble et al., ( 2010**) also reported decrease plasma vitamin D-levels in children with autism and considerable improvement in several patients with vitamin D treatment. The presence of high oxidative stress and chronic inflammation is a characteristic of ASD ***(***[***Deth et al., 2008***](http://www.ncbi.nlm.nih.gov/pubmed/18031821)***)***. Vit. D increases brain levels of powerful natural antioxidant glutathione and down-regulates production of inflammatory cytokines in the brain, which have consistently been associated with cognitive impairment ***(Moore et al., 2005)***.

**Assessment of vitamin D Status**

Vitamin D status is assessed by measuring the prohormone 25(OH)D, which is an indicator of nutritional intake and endogenous synthesis, rather than function.

It is the most stable and plentiful metabolite of vitamin D in human serum, 25(OH) D has a half-life of about 5 weeks, making it the most suitable indicator of vitamin D status ***(Timpini et al., 2011)***. The term vitamin D insufficiency has been used to describe suboptimal levels of serum 25(OH)D that may be associated with other disease outcomes. Precisely defining vitamin D deficiency or insufficiency on the basis of 25(OH) D values is still a matter of much debate. A cut off value of 30 ng/mL is sometimes used for optimal vitamin status ***(Thacher et al., 2011)***.

# Vitamin D Binding Protein

The vitamin D binding protein (DBP), originally known as the Group-specific component (Gc-globulin), is a multifunctional serum glycoprotein synthesized in large quantities by hepatic parenchymal cells and secreted into the circulation as a monomeric mature peptide of 458 residues and three structural domains ***(Daniel J. et al., 2018).***

DBP gene is a member of a multigene family that includes albumin (*ALB*)*,* α-fetoprotein (*AFP*)*,* and α-albumin/afamin (*AFM*), linked in the following order*:* centromere*–DBP–ALB–AFP–AFM-*telomere ***(Naidu S. et al., 2011).*** Among the genes affected by environmental factors are those associated with vitamin D binding protein (DBP**) *(***[*Rahimi*](https://dmsjournal.biomedcentral.com/articles/10.1186/s13098-019-0422-1#auth-1) ***et al., 2019)*.**

The vitamin D binding protein (DBP) is the major plasma carrier for vitamin D and its metabolites, but also is the precursor to the immunomodulatory protein.

**Vitamin D bound to DBP** is transported within the organism, facilitating access of vitamin D to various tissues and cell types as well as regulating the total amount of vitamin D available for the organism. Although the majority of vitamin D is bound to DBP, vitamin D can also be found bound to albumin and chylomicrons (lipoprotein particles) at lower levels and affinity. Binding to chylomicrons occurs mostly during the initial influx of vitamin D obtained by dietary or oral supplementation routes. Vitamin D produced by skin exposure to UV is quickly bound by DBP. This difference has been suggested to account for the longer lasting increase in vitamin D levels as a result of solar exposure.

**Because vitamin D is vital to the organism,** DBP’s main function is to retain vitamin D for the organism and make it available to tissues for usage. From this starting point, conceptually, there are several mechanisms by which tissues within the organism could access the vitamin D supply. Tissues could receive vitamin D by diffusion of the free ligands across membranes. In this scenario, the 25D crosses the membrane and is converted into 1,25D by the action of cytochrome within the cell to drive vitamin D regulated gene expression (local or intracrine action). In the case of 1,25D, vitamin D regulated gene expression would occur directly in line with the classic mode of endocrine action of hormones. Vitamin D could also enter tissues while still attached to DBP through active-receptor-mediated uptake to accomplish vitamin D regulated gene expression regulation.

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