Gestational vitamin D deficiency: long-term effects on the brain

Cathy W Levenson and Silvia M Figueirôa

Gestational vitamin D deficiency causes permanent changes in the developing rat brain. Not only does it alter brain gene and protein expression, deficiency disrupts the balance between neuronal stem cell proliferation and programmed cell death in the offspring. These data are particularly relevant in light of new work showing a high prevalence of vitamin D deficiency in humans.

It is generally believed that the requirements for the essential nutrient vitamin D can be met by the diet or de novo synthesis from ultraviolet light exposure, typically from sunlight. However, recent studies have suggested a surprisingly high prevalence of vitamin D deficiency, particularly in pregnant women and newborns, even in populations with normal sun exposure.

Although vitamin D deficiency is associated with bone abnormalities such as rickets, it is now recognized that the potential dangers of low vitamin D status are wide-ranging. As illustrated in a recent supplement to this journal, vitamin D plays a role in many molecular processes that govern cellular proliferation, differentiation, and survival as well as modulation of a variety of hormonal mechanisms. Thus, hypovitaminosis D can lead to increased risk of cancer, tuberculosis, diabetes mellitus, coronary heart disease, hypertension, rheumatoid arthritis, and obesity. Additionally, there is now a growing body of literature suggesting that low levels of vitamin D during development may contribute to disruptions in the development of the central nervous system.

VITAMIN D IN THE BRAIN

For many years it was believed that only the kidney was able to synthesize the active, hormonal form of vitamin D, dihydroxyvitamin D (1α,25(OH)₂-D₃). We now know, however, that the human and rodent brain express 1α-hydroxylase, the enzyme responsible for the hydroxylation of 25-hydroxyvitamin D to active 1α,25-hydroxyvitamin D. Furthermore, the brain catabolizes 1α,25(OH)₂-D₃ through expression of the gene that codes for 25-hydroxyvitamin D 24-hydroxylase.

Not only is the brain able to synthesize its own active form of vitamin D, it also expresses the nuclear vitamin D receptor (VDR). This member of the steroid-thyroid nuclear receptor family is responsible for the genomic effects of vitamin D. It is widely expressed in the human and rodent cortex, cerebellum, mesopontine area, diencephalon, spinal cord, amygdala, hypothalamus, and hippocampus, with particularly strong nuclear staining in CA1, CA3, and CA4. As illustrated in Figure 1, the hormonal form of vitamin D (1α,25(OH)₂-D₃) enters cell nuclei where it associates with the VDR. VDR, in turn, can form heterodimers with the retinoic acid receptor, RXR. Together, this complex of two nutrients (vitamin D in the form of 1α,25(OH)₂-D₃ and vitamin A in the form of 9-cis-retinoic acid) and their respective nuclear receptors (VDR and RXR) bind to specific sequences of DNA known as vitamin D response elements (VDRE). Binding of this complex to VDREs in the 5′-flanking region of vitamin D responsive genes, in concert with other nuclear factors, results in the regulation of gene transcription both in neurons and neuronal precursor cells.

Affiliations: CW Levenson and SM Figueirôa are with the Program in Neuroscience and Department of Nutrition, Food and Exercise Sciences at Florida State University, Tallahassee, Florida, USA.

Correspondence: CW Levenson, Program in Neuroscience and Department of Nutrition, Food and Exercise Sciences, 237 Biomedical Research Facility, Florida State University, Tallahassee, FL 32306-4340, USA. E-mail: levenson@neuro.fsu.edu, Phone: +1-850-644-4122, Fax: +1-850-644-0989.

Key words: apoptosis, brain, stem cells, schizophrenia, VDR, vitamin D

Emerging Science
Immediate postnatal effects

A large number of genes involved in the process of cellular proliferation, differentiation, and survival are regulated by both vitamin D and retinoic acid. Thus, it is not surprising that vitamin D plays a significant role in brain development. Pups born to dams that were vitamin D deficient 6 weeks before mating and during the length of pregnancy had longer, thinner brain cortices with larger lateral ventricular volumes. Interestingly, developmental vitamin D deficiency increased cellular proliferation in the brain and reduced the amount of apoptotic cell death that is normally associated with neuronal differentiation. These findings are consistent with work showing that developmental vitamin D deficiency causes reductions in p75NTR, a neurotrophic receptor associated with programmed cell death during development, and the deregulation of a variety of apoptosis genes, including Bcl-2, and a number of cyclin genes, which regulate the cell cycle and cellular proliferation during development.

Cui et al. sought to test the hypothesis that vitamin D deficiency impairs neurogenesis during development. The subventricular zone (SVZ) that surrounds the lateral ventricles is a region rich in neuronal stem cells. We now know that this region of the brain has the ability to produce new neurons, not just during the developmental period, but throughout the lifespan. Thus, it is an excellent site for studying the role of vitamin D in stem cell proliferation and neurogenesis. Using a cultured neurosphere assay from SVZ tissue collected on the day of birth, the investigators observed higher proliferative rates in cells isolated from the offspring of vitamin D-deficient dams compared to offspring from vitamin D-replete controls. As expected, because 1α,25(OH)2-D3 has a well-known antiproliferative effect on cells, the addition of 1α,25(OH)2-D3 to control cultures resulted in decreased neurosphere formation. However, surprisingly, 1α,25(OH)2-D3 treatment of cultures derived from pups of vitamin D-deficient dams had no effect on neurosphere proliferation, suggesting that vitamin D deficiency during gestation causes changes in the 1α,25(OH)2-D3 responsiveness of brain cells in the newborn.

Initially, the investigators hypothesized that 1α,25(OH)2-D3 supplementation of brain cell cultures from the pups of vitamin D-deficient dams was ineffective because gestational vitamin D deficiency in the dams caused some impairment of VDR expression in their offspring. However, when they tested this hypothesis directly, they found that neither brain VDR mRNA levels nor protein levels in the offspring of vitamin D-deficient dams were different than in pups from vitamin D-sufficient control dams. This led to the speculation that developmental vitamin D deficiency permits the expression of VDR, but that the receptor is somehow rendered non-functional in developmentally vitamin D-depleted animals.

Long-term effects

Unfortunately, it appears that the effects of gestational vitamin D deficiency may result in long-term, or even permanent, alterations in the brain of the offspring that
cannot be overcome by postnatal vitamin D supplementation. When Feron et al.\textsuperscript{14} reintroduced vitamin D either at birth or during weaning to pups born to vitamin D-deficient dams, many of the alterations observed in the vitamin-deficient neonatal brains still persisted even at 10 weeks of age. Ventricles sizes remained abnormal, as did many molecular indices, including regulation of a variety of genes that code for cytoskeletal proteins, neurotrophins, and neurotransmitter receptors.\textsuperscript{14}

### MOLECULAR MECHANISMS

Initial mechanistic studies were recently reported using gene array and protein expression profiling in adult rats that were vitamin D-deprived during gestation.\textsuperscript{15,16} Using an Affymetrix platform designed to study over 1300 genes involved in the development of the nervous system, Eyles et al.\textsuperscript{15} found 74 different functional genes that were differentially regulated greater than twofold in 10-week-old offspring of vitamin D-deficient dams that had been kept on a vitamin D-deprived diet and special lighting condition 6 weeks before mating and during pregnancy. Most of these genes (82\%) were downregulated compared to controls. Functional analysis of the regulated mRNAs showed that they coded for cytoskeletal, mitochondrial, and synaptic proteins. Of particular interest were several differentially regulated genes associated with the cellular proliferation and growth, such as the following: Gadd45a, a growth arrest and DNA-damage inducible gene; Mapk9, a mitogen-activated gene; Gas5, a growth arrest specific gene; and insulin-like growth factor 1.\textsuperscript{15}

A related study used the same method of dietary deficiency prior to mating and throughout pregnancy to induce developmental vitamin D deficiency and then employed two-dimensional electrophoresis and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy to examine differential protein expression in the frontal cortex and hippocampus of 10-week-old rats.\textsuperscript{16} Like the oligonucleotide array data, the proteomic analysis resulted in the identification of differentially regulated cytoskeletal, mitochondrial, and neuronal transmission proteins. While there was some tandem regulation of both mRNA and protein expression, such as the downregulation of both glial fibrillary acidic protein (GFAP) mRNA and protein abundance, there was surprisingly little overlap between the genomic and proteomic analyses. This may be due, at least in part, to the fact that the mRNA analysis was conducted on whole brain samples, while the proteomic analysis was conducted specifically in frontal cortex and hippocampus. Despite this limitation, it is clear from these studies that the developing brain is susceptible to gestational vitamin D deficiency and that inadequate vitamin D supply during this critical developmental period may permanently alter the molecular regulation of brain function.

### VITAMIN D DEFICIENCY AND NEUROPATHOLOGY

Together, these and other data recently reviewed by McCann and Ames,\textsuperscript{17} suggest there is a critical role for vitamin D in brain development. Less clear is the long-term neuropathological and behavioral implications of developmental deficiencies. For example, while one study reported increased anxiety in knockout mice lacking the vitamin D receptor gene,\textsuperscript{18} another found no differences in anxiety-like behaviors in the elevated plus arm maze test or in learning and memory compared to wild-type litter mates.\textsuperscript{19} Vitamin D receptor knockout mice, did, however, appear to have impairments in habituation to both a novel open field environment and in the acoustic startle response.\textsuperscript{19}

Interestingly, epidemiological studies have indicated an increased risk of schizophrenia\textsuperscript{20} in populations living at higher latitudes and in individuals born during winter months, areas and times with less sun exposure and cutaneous vitamin D production. Thus, while there is little doubt that schizophrenia is a multifactorial disorder, with environmental, genetic, and possibly epigenetic causes, it is possible that there could be a link between the occurrence of this neurological disorder and low gestational vitamin D levels. Moreover, this concern may have relevance to disparities in the rates of schizophrenia in African Americans\textsuperscript{21} and other darker skinned people, given that over 40\% of African American women of childbearing age may be vitamin D deficient\textsuperscript{22} and in some female populations with high skin pigmentation and low sun exposure, the prevalence of vitamin D deficiency may reach 80\%.\textsuperscript{23,24}

In addition to a possible epidemiological link between vitamin D status and risk of certain neurological diseases, there is also structural and molecular evidence suggesting an association between this disorder and developmental vitamin D deficiency. For example, enlargement of the lateral ventricles seen in vitamin D deficiency is also a common pathophysiological finding in patients with schizophrenia.\textsuperscript{25} Additionally, there are reports of decreased plasma levels of nerve growth factor levels in both vitamin D deficiency and schizophrenia.\textsuperscript{26}

Examination of the genomic and proteomic work in adult rats born to vitamin D-deficient dams revealed that many of the molecules found to be dysregulated in this animal model were also disrupted in patients with schizophrenia.\textsuperscript{14-16} For example, microarray analysis of prefrontal cortex from schizophrenic subjects revealed a consistent and robust decrease in synapsin II mRNA that codes for a protein involved in the regulation of synaptogenesis and neurotransmitter.\textsuperscript{27} Several other
studies have implicated polymorphisms in the synapsin II gene in the etiology of schizophrenia. These findings are particularly interesting given that synapsin II mRNA and protein levels are both reduced in the brains of adult rats that were deprived of vitamin D during development.

CONCLUSION

The work reviewed here suggesting a link between gestational vitamin D status, long-term changes in brain gene expression, and altered stem cell proliferation make this an interesting example of a nutrient-gene interaction that could affect the brain and have long-term physiological and behavioral consequences. Clearly more work is needed to develop a comprehensive understanding of the cellular and molecular mechanisms in the developing brain that are regulated by vitamin D. For example, future work will be needed to test the hypothesis that early deficiency impairs VDR transcriptional activity by examination of DNA binding, ligand binding, and heterodimer formation. Studies will also be needed to determine the extent to which these changes persist into adulthood after repletion and the degree to which they alter behavior and contribute to adult neurological diseases such as schizophrenia.

REFERENCES

Copyright of Nutrition Reviews is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.