Maternal Vitamin D Deficiency and Early Childhood Health Outcomes Including Autism Development

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MATERNAL VITAMIN D DEFICIENCY AND EARLY CHILDHOOD HEALTH OUTCOMES INCLUDING AUTSIM DEVELOPMENT

by

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Approved:

UTAH STATE UNIVERSITY
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Abstract

Many studies have shown that vitamin D deficiency during pregnancy is associated with a variety of adverse maternal and pediatric outcomes. Disease outcomes that have been observed in pregnant women who are vitamin D deficient include increased risk of C-section, preeclampsia, bacterial vaginosis, and gestational diabetes. In children born to deficient mothers, increased rates of childhood asthma, type 1 diabetes, low birthweight, and autism spectrum disorder (ASD) have been observed. Although there is much evidence to support these correlations, much is yet to be understood regarding the etiology of these outcomes. This paper specifically examines the relationships between risk factors for vitamin D deficiency and development of ASD. These relationships provide strong justification for the studies that are being conducted in the USU Immunogenetics Lab as well as proposals for future investigations. Current work in the Immunogenetics lab includes analysis of single-nucleotide polymorphisms (SNPs) in the vitamin D receptor gene of individuals with ASD and their immediate families. Additionally, a recently proposed project in collaboration with Intermountain Healthcare (IHC) will also seek to find correlation between pre-conception vitamin D levels with maternal health outcomes (i.e. gestational diabetes mellitus, preeclampsia) and early childhood outcomes (i.e. childhood asthma, type 1 diabetes, low birth weight, and autism). Based on increasing evidence, and arguments presented in this paper, we expect that one or more of these outcomes will be significantly associated with insufficient or deficient vitamin D levels prior to conception. Although there are several outcomes of interest listed in the IHC study, the relationships between risk factors for vitamin D deficiency and ASD receive the most attention in this paper, due to their unique empirical consistencies.

1 Since definitions of vitamin D insufficiency and deficiency vary, we are following the ARUP laboratory’s standards which are comparable to the Endocrine Society’s standards (See Figure 7).
Acknowledgements

I would like to thank my faculty mentors including Thayne Sweeten, Crescencio López and Kim Sullivan for their continued support and endless patience. It has been a pleasure to work with each of them, and I am grateful for what I have learned from each of them. I am also grateful for the honors faculty and staff, especially Chelsea Gensel and Lisa Hunsaker, who were so kind when I was unsure of myself. Lastly, I would like to thank my sweet parents for encouraging me and supporting me in all my pursuits.
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**Figure 7**
This table shows deficiency and insufficiency definitions according to ARUP Laboratories and Endocrine Society standards.

<table>
<thead>
<tr>
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<th>ARUP Laboratories</th>
<th>Endocrine Society</th>
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<tr>
<td>Deficient</td>
<td>&lt; 20 ng/ml</td>
<td>0-20 ng/ml</td>
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<td>Insufficient</td>
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<td>Sufficient</td>
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<td>Toxic</td>
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Vitamin D is an essential nutrient for optimal health with a diversity of roles. Unlike other necessary vitamins, vitamin D is a hormone. Most vitamin D is produced in human skin when subjected to UV light from the sun. Although it can be acquired through dietary sources, these are relatively inconsequential in comparison to the amount of vitamin D that can be produced from 15 minutes of UV light exposure\(^2\). Despite the convenience of being able to produce sufficient vitamin D with daily exposure to UV light, prevalence of hypovitaminosis D has reached pandemic proportions. Moreover, irrefutable evidence has demonstrated that deficient vitamin D serum levels in the prenatal environment are associated with a variety of adverse maternal and pediatric health outcomes. Maternal health outcomes that have been associated with vitamin D sufficiency or insufficiency include: preeclampsia\(^3\), gestational diabetes mellitus\(^4\), bacterial vaginosis\(^5\), and increased risk of C-section\(^6\). Early childhood outcomes associated with low maternal vitamin D levels include: childhood asthma\(^7\), type 1 diabetes\(^8\), low birth weight\(^9\), and autism spectrum disorder (ASD)\(^10\). These findings suggest that risk factors for vitamin D deficiency may also be potential risk factor for the afore-mentioned health outcomes. These correlations have provided some potential insights into the elusive etiology of autism. Current research in the Immunogenetics Lab is focused on determining if there are abnormal ratios of genetic single-nucleotide polymorphism frequencies of the vitamin D receptor (VDR) gene in children with ASD and their immediate family members (i.e. parents and siblings). Additionally, a recently proposed project in collaboration with Intermountain

\(^{2}\) See Figure 1
\(^{3}\) (Bodnar et al. 2007)
\(^{4}\) (Krakowiak et al. 2012)
\(^{5}\) (Bodnar, Krohn, and Simhan 2009)
\(^{6}\) (Merewood et al. 2009)
\(^{7}\) (Hornsby et al. 2018)
\(^{8}\) (Miettinen et al. 2017)
\(^{9}\) (Bodnar et al. 2010)
\(^{10}\) (Cannell 2008)
Healthcare (IHC) will also seek to find correlation between pre-conception vitamin D levels with maternal health outcomes (i.e. gestational diabetes mellitus, preeclampsia) and early childhood outcomes (i.e. childhood asthma, type 1 diabetes, low birth weight, and autism). Due to the increasingly consistent association between maternal vitamin D deficiency and development of ASD, this discussion will give priority to this outcome of interest. Furthermore, the consistency of this relationship lends validity to the hypothesis that development of ASD in offspring will display a significant correlation with risk factors associated with maternal vitamin D deficiency.

Many factors contribute to vitamin D levels. It is important to explore what these factors are and how they affect vitamin D levels to draw connections between these potential risk factors and the disease outcomes of interest. Age has been shown to be a contributing factor to vitamin D deficiency. There are a variety of well-founded explanations for increased risk of vitamin D deficiency among the elderly population. For one, there is an observed age-dependent decrease in the epidermal concentrations of previtamin D₃ (7-dehydrocholesterol) which leads to an overall decrease in the skin’s ability to photosynthesize vitamin D (MacLaughlin and Holick 1985). A comparison study on the amount of previtamin D₃ produced in the skin from the 8- and 18-yr-old subjects and the amount produced in the skin from the 77- and 82-yr-old subjects revealed that aging can cause a twofold decrease the capacity of the skin to produce provitamin D₃ (MacLaughlin and Holick 1985). Another reason for the reduction in circulating vitamin D levels is a decrease in renal function that occurs with age. One study infused women of various ages with PTH (parathyroid hormone) for 24 hours and recorded subsequent serum vitamin D levels. They found that the following increase in serum 1,25(OH)₂D was about 50% lower in the older women. This demonstrated decreased renal responsiveness to PTH with age and showed that serum 1,25(OH)₂D levels decrease as a result of an age-related decline in renal function.

See Figure 3
Another possible explanation for this phenomenon is that aging may affect the intestinal concentration of vitamin D receptor (VDR) and thereby lead to decreased calcium absorption, as demonstrated in aging rats (Horst, Goff, and Reinhardt 1990). This is related to the increased incidence of osteomalacia that is observed among the elderly since 1,25(OH)₂D is responsible for calcium regulation and absorption. Various studies have been performed to assess human concentrations of VDR in the intestine across different ages with mixed results. However, it is apparent that increasing age is a significant contributor to vitamin D deficiency.

Perhaps the most unique quality of vitamin D is its ability to be photosynthesized by human skin upon exposure to UV light. Irradiation of human skin with ultraviolet B (280-320 nm) initiates the photochemical conversion of 7-dehydrocholesterol to vitamin D₃ (Lehmann and Meurer 2010). The effectivity of UVB on production of provitamin D₃ is influenced by chromophores, which include an atom or group whose presence is responsible for the color of a compound, which refers to melanin, DNA, RNA, proteins and 7-Dehydrocholesterol (7-DHC). (Lehmann and Meurer 2010). Studies have shown that the production of vitamin D₃ by human skin is decreased in individuals with higher concentrations of melanin, a skin pigment which is responsible for the presentation of darker skin tones. One study experimentally determined the effect of increased skin pigment on the cutaneous production of vitamin D₃ (Clemens et al. 1982). The study included three fair-skinned Caucasian volunteers and two African American volunteers. All individuals in the study were exposed to one standard dose of UV light. After exposure, blood samples were drawn on each of the individuals and vitamin D levels were determined and compared to base vitamin D readings. The results showed that 1 minimal erythemal dose of UVR in the Caucasian volunteers greatly increased serum vitamin D concentrations by up to 60-fold 24-48 hours after exposure, whereas this dose did not
significantly change serum vitamin D concentrations in African American subjects (Clemens et al. 1982). These results provided evidence that increased melanin content decreases the ability of human skin to produce vitamin D. A more current study concluded that 80% of the response of 25(OH)D levels to UVB light is dependent on skin pigmentation and the amount of UVB given (Armas et al. 2007). According to an analysis of data collected by the National Health and Nutrition Examination Survey, the overall prevalence rate of vitamin D deficiency among US adults was 41.6%, with the highest rate seen in blacks (82.1%), followed by Hispanics (69.2%) (Forrest and Stuhldreher 2011). Another study that looked at vitamin D deficiency among healthy US teenagers found similar results. They discovered that prevalence was highest in African American teenagers and during winter, although hypovitaminosis D seemed to be common across sex, season, and ethnicity (Gordon et al. 2004). Increased skin pigmentation reduces individual ability to produce adequate levels of vitamin D, but the amount of sunlight that touches our globe affects entire populations.

Seasonal sunlight patterns seem to have a profound effect on circulating vitamin D levels. For example, northern latitudes receive less sunlight during the winter causing vitamin D levels to shift in accordance with the seasons. A study conducted in southern Florida, an area characterized by relatively consistent sunny weather, tested the vitamin D levels of adults after winter and after summer. The mean (±SD) winter 25(OH)D concentration was 24.9 ± 8.7 ng/ml (62.3 ± 21.8 nmol/liter) in men and 22.4 ± 8.2 ng/ml (56.0 ± 20.5 nmol/liter) in women, and in the 99 subjects tested for vitamin D levels at the end of summer, the mean 25(OH)D concentration was 31.0 ± 11.0 ng/ml (77.5 ± 27.5 nmol/liter) in men and 25.0 ± 9.4 ng/ml (62.5 ± 23.5 nmol/liter) in women (Levis et al. 2005a). These statistically significant results include a 14% summer increase in 25(OH)D concentrations in men and a 13% increase in women (Levis
et al. 2005b). Due to more consistent sunlight in southern latitudes, we would expect the results to be more dramatic in northern latitudes due to greater variance in cyclic light patterns. A vitamin D insufficiency study that was performed in Canada observed an anticipated rise in serum 25(OH)D from a mean of 57.3 (standard deviation [SD] 21.3) nmol/L in the winter to 62.9 (SD 28.8) nmol/L in spring ($p = 0.001$) and 71.6 (SD 23.6) nmol/L in summer ($p < 0.001$), with a subsequent decline to 52.9 (SD 17.2) nmol/L in the fall ($p = 0.008$) (Rucker et al. 2002). Given the lack of sunlight and the larger zenith angle\(^{12}\) that characterizes northern winters, it has been hypothesized that dark-skinned minorities are at greater risk of deficiency and insufficiency as they migrate to northern latitudes. An interventional study that tested the vitamin D status of pregnant women from non-European ethnic minorities in Southern Wales concluded that there existed a high incidence of subnormal vitamin D levels in women from ethnic minorities (Datta et al. 2002). Another study involving South Asian populations in the UK suggested that vitamin D deficiency could be correlated with development and progression of chronic diseases, including type 2 diabetes (Lowe and Bhojani 2017). Furthermore, multiple studies have demonstrated that dark-skinned immigrants in countries with relative lack of sun have an elevated autism prevalence (Fernell et al. 2015). The combined effects of skin pigmentation, seasonal sunlight variation and latitude continues to correspond with existing data that vitamin D deficiency and correlated health outcomes appear to be most prevalent among dark-skinned minorities.

Hypovitaminosis D has been associated with a variety of adverse conditions, especially in consideration of maternal and early childhood health outcomes. The maladies associated with vitamin D deficiency in the mother that are of greatest interest in our collaborative data study

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\(^{12}\) (“SolarZenith.Pdf’ n.d.) Explains how to calculate the zenith angle from your latitude at noon. The formula for calculating the zenith angle at noon on December 21 (winter solstice) is “zenith angle = latitude + 23.5”. Hence, northern latitudes have a higher zenith angle in winter months.
with IHC include: preeclampsia and gestational diabetes mellitus. Disease outcomes of interest in children involved in this study will include: low birth weight, childhood asthma, type I diabetes and ASD. Each of these outcomes has been correlated in multiple studies with maternal vitamin D deficiency\textsuperscript{13,14,15,16,17}. Of the outcomes of interest, development of ASD in early childhood is the most relevant to prior discussions on vitamin D deficiency across age, race, geographical area and season. Additionally, the diverse roles of vitamin D in the immune system, nervous system and calcium metabolism also appear to affect development of ASD.

The observed connection between ASD and deficient gestational vitamin D levels, justifies the expectation that risk factors for vitamin D deficiency in the mother would also be associated with increased rates of autism in offspring. A historical birth cohort study conducted in Northern California examined the relationship between maternal and paternal age and risk of autism. Risk of ASD increased significantly with each 10-year increase in maternal age (adjusted risk ratio (RR), 1.31; 95% confidence interval [CI], 1.07-1.62) and paternal age (RR, 1.28; 95% CI, 1.09-1.51) (Croen et al. 2007). Thus, each 10-year increase in a mother’s age increased the risk of having an autistic child by 1.31 times. Adjusted risk ratios for both maternal and paternal age were elevated for children with autistic disorder (maternal age: RR, 1.18; 95% CI, 0.87-1.60; paternal age: RR, 1.34; 95% CI, 1.06-1.69) (Croen et al. 2007). Studies of paternal age effects on offspring ASD incidence have mixed results, however maternal age seems to consistently display a non-linear influence on ASD incidence in offspring. A similar study conducted on Swedish medical records showed that risk of autism among mothers <29 years old was about the same,

\textsuperscript{13} (Hornsby et al. 2018) Vitamin D supplementation during pregnancy influences the immune system of the neonate, which can contribute to protection from asthma-related outcomes in early life.
\textsuperscript{14} (Khalessi et al. 2015) Maternal Vitamin D deficiency may increase the risk of low birth weight in neonate.
\textsuperscript{15} (Miettinen et al. 2017) Results of this study show that the in utero environment including maternal vitamin D metabolism should be important lines of investigation when searching for factors that lead to genetic programming of type 1 diabetes.
\textsuperscript{16} (Garipardic et al. 2017) Both ADHD and ASDs accompany vitamin B12 and D deficiency.
\textsuperscript{17} (Vinkhuyzen et al. 2018) Maternal vitamin D deficiency is correlated with autistic outcomes in children.
but risk increased after age 30, with an odds ratio (OR) of 1.75 [95% (CI): 1.63–1.89] at ages 40–45 (Idring et al. 2014). This odds ratio of 1.75 indicates that a child with ASD is 1.75 times more likely to have been born to mother ages 40–45 years old. They also concluded that an increase of \( n \) years in maternal age has greater implications for ASD risk than a similar increase in paternal age (Idring et al. 2014). Further testing will need to be done to determine to what extent paternal age affects childhood outcomes, and the reasoning behind the stronger influence of maternal age on offspring ASD incidence. However, the data does support the hypothesis that hypovitaminosis D risk factors, such as increased age, also affect ASD development when those risk factors are present during gestation.

The same logic can be applied to race and corresponding melanin concentration. If increased melanin concentration is a risk factor for vitamin D deficiency, and vitamin D deficiency is linked to increased risk of ASD, it would be appropriate to see increased prevalence of ASD across races with increased melanin content. It would also be reasonable to expect intermediate levels of prevalence of ASD among races with intermediate levels of skin pigmentation. Studies have indicated that there are racial differences in ASD prevalence\(^{18}\). However, many of the prevalence rates do not follow the hypothesized expectations. According to the Autism and Developmental Disabilities Monitoring Network, when data from 14 sites in the U.S. were combined, the estimated prevalence among non-Hispanic white children (12.0 per 1,000) was significantly greater than that among non-Hispanic black children (10.2 per 1,000) and Hispanic children (7.9 per 1,000) (“Prevalence of Autism Spectrum Disorders — Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008” 2012). One explanation for these unexpected differences is that socioeconomic status seems to have an affect

on prevalence. Generally speaking “Black children are much more likely to be in the low-
socioeconomic group than white children” says Maureen Durkin, lead researcher and professor
and interim chair of population health sciences at the University of Wisconsin-Madison (“Race,
Class Contribute to Disparities in Autism Diagnoses” 2017). It is thought that perhaps lower
socioeconomic classes do not seek out medical diagnoses as readily as the higher socioeconomic
classes. This could be due to financial instability, cultural distrust for medical institutions,
language barriers, and lack of concern or awareness of autism. These reasons could also apply to
the observed lack of participation in prevalence studies among ethnic and racial minorities.19. In
reports of studies conducted in the early 1980s, “investigators concluded that the differences in
autism prevalence among socioeconomic classes was likely due to a social class bias in access to
diagnostic services and treatment in the clinic populations from which study participants were
drawn” 20,21,22 (Bhasin and Schendel 2007). Interestingly, surveillance data from the U.S. Census
Bureau and the U.S. Centers for Disease Control and Prevention’s (CDC) Autism and
Developmental Disabilities Monitoring Network revealed that the rate of autism among black
children in the high socioeconomic group was higher than that among white or Hispanic children
between 2002 and 2010 (“Race, Class Contribute to Disparities in Autism Diagnoses” 2017).
This suggests that perhaps autism prevalence studies are not getting an accurate measure of the
true prevalence among the lower socioeconomic classes and the ethnicities that are often
associated with those classes. In essence, they are not finding prevalence of autism across races,
but prevalence of diagnosis.

19 (Bradley et al. 2018) Study found that odds of continuing the study to completion were significantly lower if the
biological mother was Black. Odds of participation were increased if the mother had higher education or provided
more than one contact number. Overall, minorities were less likely to participate.
20 (Schopler, Andrews, and Strupp 1979)
21 (“Social Class Distribution of Fathers of Children Enrolled in the Iowa Autism Program | SpringerLink” n.d.)
Results suggest that if services become better known and readily available, then no differences in social class
distribution between autistic and nonautistic groups will occur.
22 (“BJP Volume 212 Issue 4 Cover and Front Matter” 2018)
Despite these complicating factors, there is evidence that there is increased prevalence of autism in Black children according to several studies. According to a study conducted on sociodemographic risk factors for autism in US metropolitan areas, the adjusted analyses revealed that among all children with autism, there exists an over twofold increased risk for autism was seen among Black children compared with White children (Bhasin and Schendel 2007). Other studies have supported the finding of increased prevalence of autism in Blacks\textsuperscript{23,24,25}. Despite mixed results on race and autism prevalence, the number of studies that have demonstrated a lack of participation among racial and ethnic minorities make it probable that many autism prevalence studies are not representative of true rates among Blacks and Hispanics. Thus, it is likely that the prevalence of autism in racial minority groups is largely underestimated.

Due to the relationship between hypovitaminosis D and geographical location, it can be rationalized that northern latitudes are likely connected to autism prevalence as well. According to a 2009 prevalence study, there is epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of autism. This was evidenced by a strong positive correlation between increasingly northern latitudes (related to wintertime solar ultraviolet B radiation) and increased rates of infantile autism disease (IAD)\textsuperscript{26} (Grant and Soles 2009). These findings are consistent with maternal vitamin D deficiency being a risk factor for IAD, possibly by affecting fetal brain development as well as possibly by affecting maternal immune system status during pregnancy (Grant and Soles 2009). A separate ecological study found that autism prevalence in the US among those aged 6–17 years in 2010 was significantly

\textsuperscript{23} (Croen et al. 2002)
\textsuperscript{24} (Hillman et al. 2000)
\textsuperscript{25} See Figure 2
\textsuperscript{26} See Figure 4
inversely correlated with solar UVB doses\textsuperscript{27} (Grant and Cannell 2013). The same study also uncovered significant positive correlations between increasing latitude and autism prevalence especially among the Black populations analyzed. A 2008 prevalence study conducted by the CDC across 14 states found that New Jersey had the highest autism prevalence and Alabama had the lowest autism prevalence, with New Jersey being the second northernmost state and Alabama being the southernmost state in the study ("Prevalence of Autism Spectrum Disorders — Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008" n.d.). Clearly there is ample evidence of a connection between the quantity of UVB light exposure at different latitudes and autism prevalence.

If the amount of UVB light exposure affects maternal vitamin D and autism prevalence, autism rates should also fluctuate on a seasonal basis as the amount of sunlight reaching the earth’s northern latitudes decreases in winter months. Many studies have shown promising birth patterns. Moreover, maternal vitamin D levels seem to correspond with this pattern. There is an observable cyclic rise and fall in maternal serum (OH)D levels that corresponds with seasonal variations in sunlight\textsuperscript{28}. One investigation on month of conception and development of ASD concluded conception in the winter season (December, January, and February) was associated with a 6\% (OR = 1.06, 95\% CI = 1.02 – 1.10) increased risk compared with summer (Zerbo et al. 2011). In an examination of the association between ASD and birth month the highest incidences of ASD were recorded among children born in May and August: (10.3 and 10.2 per 1000, respectively), while the lowest incidences were in January and February (7.6 and 8.1 per 1000, respectively) (Shalev, Solt, and Chodick 2017). Another investigation that was conducted in Israel, supported previous studies when researchers claimed a significant increase was observed

\textsuperscript{27} See Figure 5
\textsuperscript{28} See Figure 6
for children born in March and August (Barak et al. 1995). These findings do not seem to correspond with investigations on month of conception and ASD development. If conception during January, February and December is associated with increased ASD rates, the corresponding birth months would be August, September and October. While August was mentioned as one of the birth months with increased ASD prevalence, September and October are not. Although this seems inconsistent with the expected results, it has been suggested that inconsistencies in the literature could be due, in part, to a wide range of study populations, geographical areas, case definitions, comparison groups and analytical approaches (Shalev, Solt, and Chodick 2017). Furthermore, preterm birth has been found to be associated with ASD with an odds ratio of 2.05 [95% CI: 1.26–3.34] and 1.55 [95% CI: 1.22–1.96] for very and moderately preterm births respectively\(^{29}\). Considering the probability that a child who has ASD was born prematurely, it is not surprising that increased ASD prevalence conception months do not precisely match with the increased ASD prevalence birth month that would be predicted based on an average 40-week\(^{30}\) gestation period. More studies will need to be done to prove the validity of this hypothesis, but current and increasing evidence is supportive of the relationship.

Existing data shows consistent correlations between vitamin D deficiency and the development of ASD, but the mechanisms behind why this is the case remain in their hypothetical stages. The Immunogenetics lab at Utah State University seeks to establish stronger conclusions on the mechanics of this relationship. Current work in the lab includes multiple assays utilizing several different restriction enzymes to determine whether there is an abnormal ratio of mutations in the vitamin D receptor (VDR) gene in children with ASD and their immediate families. Based on previous discussions and evidence, we hypothesize that there will

\(^{29}\) (Buchmayer et al. 2009)

\(^{30}\) (Services n.d.) The unborn baby spends around 38 weeks in the uterus, but the average length of pregnancy, or gestation, is counted at 40 weeks.
be an increased frequency of single nucleotide polymorphisms in the VDR gene among autistic children and their immediate family members. Additionally, a proposal was recently submitted to IHC to perform a retrospective data study of over 7,000 medical records that will provide vitamin D levels of women within one year prior to pregnancy and will link these values with pregnancy outcomes and early childhood outcomes. We expect that one or more of these outcomes will display a significant correlation between pre-pregnancy vitamin D deficiency and adverse health outcomes for both mother and child. The goal of these efforts is to provide foundational data for further collaboration in clinical trials involving vitamin D supplementation testing. Ultimately, clinical trials have the experimental power to show cause and effect. Powerful evidence will be necessary to determine adequate supplementation dosages during pregnancy and before conception. The hypotheses and aims of these investigations are based on prior discussions of this paper as well as more recent findings on the diversity of roles that vitamin D performs in the human body. These metabolic roles include neurological development, calcium homeostasis, and immune regulation.

The role of vitamin D in regulation of calcium levels in the blood is related to neurological health. When blood calcium levels drop, the parathyroid gland begins to secrete parathyroid hormone (PTH). PTH travels in the blood stream to the bones where it triggers a release of some of the stored calcium of the bone tissues. PTH also signals the kidneys to produce more 1,25(OH)D which, in turn, activates the absorption of more calcium through the digestive tract ("Parathyroid Glands and Vitamin D" 2010). This mechanism is regulated by a negative-feedback loop to prevent overproduction of PTH. The decreased ability to absorb calcium that accompanies vitamin D deficiency provides an etiological explanation of rickets in children and osteoporosis in adults who are vitamin D deficient (Wrzosek et al. 2013). Since
vitamin D deficiency reduces bone health, decreased bone health in autistic children would be expected. One study “determined whether bone mineral density (BMD) is lower in boys with autism spectrum disorders (ASD) than controls...[the researchers] concluded that BMD is lower in peripubertal boys with ASD and may be associated with impaired vitamin D status and lower exercise activity” (Neumeyer et al. 2015). Calcium balance is also necessary for normal nerve function. One of the neuroprotective roles of vitamin D involves the synthesis of proteins that bind calcium (Ca2+) ions and thereby maintain cellular calcium homeostasis, which is necessary for nerve impulse transmission (Wrzosek et al. 2013). The influence of vitamin D in the central nervous system (CNS) also extends to neurotrophin production and release, neuromediator synthesis, intracellular calcium homeostasis, and prevention of oxidative damage to nervous tissue. The specific effects of hypovitaminosis D on neurological function its subsequent effect on autism development have yet to be explored in depth, but accumulating evidence favors these hypotheses.

Hypovitaminosis D is also correlated with decreased immune regulation, leading to increased autoimmunity and increased susceptibility to infection (Aranow 2011). This is attributed to the presence of VDRs on the surface of immune cells such as B cells, T cells and antigen presenting cells (Aranow 2011). It has been suggested that mothers who are vitamin D deficient have overresponsive immune systems that are much more likely to attack the CNS of

31 (Wrzosek et al. 2013) The influence of the active form of vitamin D on the nervous system is associated with modifying the production and release of neurotrophic factors such as nerve growth factor (NGF), which is essential for neuron differentiation, as well as increasing the levels of glial cell line-derived neurotrophic factor (GDNF).
32 (“Vitamin D and Omega-3 Improve Mental Health by Regulating the Synthesis of Serotonin” n.d.) Recent studies reveal that vitamin D and omega-3 fatty acids can prevent and ameliorate a number of neurological ailments such as depression, ADHD, bipolar disorder, autism, and schizophrenia. There is evidence suggesting that vitamin D and omega-3 fatty acids work by controlling the synthesis of serotonin in the brain.
33 (Veldurthy et al. 2016) Vitamin D is the principal factor that maintains calcium homeostasis. Increasing evidence indicates that the reason for disturbed calcium balance with age is inadequate vitamin D levels in the elderly.
34 (Wrzosek et al. 2013) Rat neuron culture studies showed that 1,25- (OH)2D3 increases glutathione levels in these cells. The reduced form of glutathione (GSH), supplied into nerve cells by astrocytes, is a fundamental antioxidant in protecting cells against reactive oxygen species and apoptosis caused by oxidation.
the developing fetus. Studies have also shown that inflammation in the CNS may be a contributor to the development of ASD symptoms. In one study, interleukin-6 (IL-6) levels—a chemical that is involved present in the inflammatory response—were significantly greater in the cerebellum of autistic subjects as compared with matched controls. These findings suggest that the “elevated IL-6 in the autistic brain could cause an imbalance of neuronal circuits through its effects on neural cell adhesion, migration and synapse formation, and contribute to the development of autism” (Wei et al. 2011). Perhaps, this inflammation began before birth with an overreactive maternal immune system.

The development of the fetal CNS typically occurs in the fifth week of gestation, which is before many women are aware that they are pregnant. This suggests that by the time many women find out they are pregnant, it could be too late to begin vitamin D supplementation as a protective measure against development of autism. For this reason, the collaborative study with IHC has been proposed to collect vitamin D levels within one year prior to pregnancy.

The vast empirical support and consistency of data supporting the relationship between risk factors for vitamin D deficiency and ASD development are unique in the field of autism research which is characterized by inconsistency. Although, the notion of vitamin D affecting autism development was proposed by Cannell in 2008, this is perhaps the first time in the field of autism research that there seems to be a consistent observable pattern for potentially understanding etiology of this broad-spectrum disorder. Current understanding of vitamin D metabolism and function has opened unanticipated doorways for research on other deleterious health outcomes associated with vitamin D deficiency during pregnancy. These findings are

35 ("Fetal Development: MedlinePlus Medical Encyclopedia" n.d.) Fetal development of the CNS occurs within the fifth week of gestation. Many women do not know that they are pregnant before the fifth week.
36 (Cannell 2008)
37 (Wang et al. 2016) This meta-analysis found that “levels of serum 25(OH) D in participants with ASD were significantly lower than controls, suggesting that lower vitamin D level might be a risk factor for ASD.”
fascinating and provide a robust foundation for justification of clinical trials. It is likely that the results of clinical trials will call for medical reform of vitamin D supplementation recommendations before and during pregnancy\textsuperscript{38}. Some pioneering scientists such as Bruce Hollis and Carol Wagner have already begun to voice the need for such changes. Time and trial will tell if prevention of autism and other adverse pregnancy outcomes is a function of public education and providing adequate vitamin D supplementation to all levels of the socioeconomic pyramid.

\textsuperscript{38} (Hollis and Wagner 2017)
Reflection

I became interested in doing research on vitamin D deficiency when I heard an in-class presentation by Bruce Hollis in fall of 2017. He and his colleagues had performed clinical trials on pregnant women and varying levels of vitamin D supplementation. The researchers had followed these women through their pregnancies and observed post-partum outcomes for both mother and child. They made several discoveries. Among them were significantly reduced risk of complications during pregnancy (i.e. preeclampsia, gestational diabetes), reduced risk of adverse childhood health outcomes (i.e. childhood asthma), and that current supplementation recommendations are not adequate for maintaining sufficient vitamin D levels during gestation. His work has been highly criticized especially among members of the medical community who have raised concerns regarding vitamin D overdose and liver toxicity. This is in part because his findings showed no adverse effects associated with as much as a 10-fold increase in current recommendations in supplementation dosage in pregnant women.

I found his study intriguing due to my desire to enter the medical field as a physician assistant and one day specialize in obstetrics, gynecology and women’s health. A couple of months after the presentation, I approached Professor Thayne Sweeten to get started on a research project. He was extremely patient with me as I became familiar with the difficulties and challenges associated with initiating a research project. Initially, I proposed a plan to collect data from Intermountain Healthcare’s database on vitamin D samples taken from pregnant women in the last 5 years. My goal was to look at vitamin D levels during pregnancy and link them to childhood health outcomes. Thayne and I drove down to IHC headquarters in Salt Lake to meet with Dr. Brent Muhlestein to help us initiate this project. He informed us that there is plenty of data we could use, and that our project outline was feasible, but would need to clarify some of
the details. Since that meeting in February we have drafted a new proposal for this project and sent it to Brent for review. We hope to continue with this project over this summer. Since this project remains in its preliminary stages, I began to shift my focus towards writing a literary review of the knowledge I have gained concerning vitamin D deficiency during gestation, presenting that information in a research setting and educating at-risk groups.

I was glad to have the opportunity to present my work in the Student Research Symposium held in the Merril-Cazier Library this spring. Normally, I tend to be nervous for presentations. However, when it was time for me to present I felt so confident in what I had learned and was so excited to talk about it that it was not scary at all. It was an enjoyable and worthwhile experience.

In addition to presenting at the Student Research Symposium, I was able to work closely with Professor Crescencio López to present this information at the Hispanic Health Fair. In my research on vitamin D deficiency, I learned that increased melanin content in the skin interferes with the biochemical process of vitamin D production. I realized that some races and ethnicities are more prone to vitamin D deficiency than others due to increased concentrations of melanin. Specifically, dark-skinned immigrants that migrate northward are particularly prone to vitamin D deficiency due to the increased melanin concentration in their skin and the relative lack of sunlight at northern latitudes. I found that Hispanics and Latinos composed the largest at-risk population for vitamin D deficiency in Cache Valley. My primary motivation for completing part of my capstone was to reach out to the Hispanic/Latino community to raise awareness on this highly preventable issue. Furthermore, in my future career as a physician assistant, I want to be able to educate people so that they can make healthy life choices. I strongly believe that many of the major health crises we face today can be resolved through education and understanding.
Many people simply are not aware that the daily choices they make can affect not only their health but their children’s health as well. Vitamin D deficiency is no exception to this multi-generational phenomenon. In addition, I feel that the best healthcare providers are those who are able to explain, in an understandable and concise manner, the importance of simple daily health choices. When these explanations are accompanied with genuine kindness and care for a person’s well-being, patients are more likely to accept the changes they need to make to better their health. I believe that every person, regardless of race or ethnicity deserves this kind of high-quality care. As a physician assistant, I hope to work in a community where I will be able to use the Spanish language to provide such care to a broader spectrum of individuals. That is why I felt the need to present what I have learned about the consequences of vitamin D deficiency during gestation and autism development in children at the Hispanic Health Fair. This project gave me the opportunity to expand my Spanish vocabulary to encompass scientific and medical terminology, and to apply that vocabulary as I presented to various individuals within the Hispanic community.

My capstone experience not only provided excellent training for future research, but it turned into a job opportunity as a lab technician in the Immunogenetics lab at USU. There, I plan to continue assisting Professor Thayne Sweeten in further research into vitamin D deficiency and development of autism in children.
Bibliography


Professional Bio

Kelsey Girardelli is a senior graduating in May 2018 with a B.S. in Biology. Over the course of her college career she maintained a 3.7 GPA while representing Utah State as a cross country and track and field athlete. She is a 4-time recipient of the Whitesides Scholar Athlete Award (2013, 2016, 2017, 2018). In 2018, she received Mountain West All-Conference Academic Honors. She is also a recipient of the Pedrozzi scholarship as of May 2016 and the Ivan G. Palmblad scholarship awarded in April 2016.

Currently, Kelsey is in the process of applying to Physician Assistant programs across the country and hopes to matriculate in fall of 2019. In the meantime, she will be working as a part-time CNA, lab technician in the Immunogenetics lab in the Center for Persons with Disabilities at Utah State, and a Behavioral Therapy Technician at Utah Behavior Services in Brigham City.