INVESTIGATION INTO THE ROLE OF SERUM VITAMIN D AND ITS CARRIER PROTEIN IN TYPE 1 DIABETES

By

LINDSEY MARIE BIERSCHENK

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2009

To my family, who taught me the value of believing in myself

ACKNOWLEDGMENTS

First, I would like to thank Dr. Mark Atkinson, for believing in me enough to accept me into his laboratory and for providing me with a place to learn the true value of scientific research. Dr. Atkinson served as a model of just how fruitful one's labor can be when you are a team player, use your moral compass, and never give up on even the loftiest of goals. Clive Wasserfall's exceptional work ethic, invaluable counsel, and enthusiastic nature proved to be both contagious and inspirational. I have much gratitude to Dr. Nancy Denslow and Dr. Sihong Song, for serving on my graduate committee and for providing me with excellent advice and support. I would like to thank my parents and sister for supporting me throughout some of the most challenging times in my life, from being diagnosed with type 1 diabetes, to pursuing my dream of finding a cure for the disease. Most of all, I would like to thank God for guiding my steps and instilling in me a 'can-do' spirit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

INVESTIGATION INTO THE ROLE OF SERUM VITAMIN D AND ITS CARRIER PROTEIN IN TYPE 1 DIABETES

By

Lindsey Marie Bierschenk

May 2009

Chair: Mark Atkinson
Major: Medical Sciences

Previous studies, largely in Northern Europe, have suggested an association between type 1 diabetes (T1D) and reduced serum 25(OH)-vitamin D levels. To ascertain whether this association was present in a solar rich region, we measured serum 25(OH)-vitamin D levels of 415 individuals in Florida, USA. Study subjects included 153 controls, 46 new-onset T1D patients, 110 established T1D patients (onset > 5 months from diagnosis), and 106 first-degree relatives of diabetic patients. UV Index climatological data was obtained to estimate average relative solar exposure for subjects at the time of sample collection. This population was expanded to measure vitamin D binding protein (VDBP) in the serum of 153 controls, 203 T1D subjects, and 116 relatives.

Serum 25(OH)-vitamin D (mean ng/mL; 95% CI) levels were similar amongst healthy controls (27.2; 23.3-31.1), new-onset T1D patients (21.8; 18.2-25.3), subjects with established T1D (27.7; 22.4-33.1), and their first-degree relatives (23.6; 21.0-26.3) (p=0.705). Suboptimal vitamin D levels (≤ 30 ng/mL) were observed in 70.1% of controls, 76.1% of new-onset T1D subjects, 68.5% of established T1D subjects, and 68.8% of relatives. Interestingly, suboptimal vitamin D levels were present in similar proportion for all groups and estimated UV exposure did not significantly impact vitamin D levels (p=0.779).

Further analysis of vitamin D status by measurement of its carrier protein, VDBP, revealed a significant difference (p=0.0055) in serum concentrations (mean µg/mL; 95% CI) between the control (528.2; 467.3-589.0) and T1D groups (424.8; 403.6-446.0). Serum VDBP concentrations were not significantly different for relatives (496.9; 410.3-583.4) compared to control or T1D subjects (p=NS). Linear regression analysis of VDBP levels versus disease duration revealed no association (r=0.0026, p=0.5158).

In sum, VDBP serum concentrations appear to be of significance in the presence of T1D while suboptimal 25(OH)-vitamin D levels were characteristic of the entire study population. Future directions would include exploring the relationship of reduced VDBP concentrations in the pre-diabetes state, as well as correlation with genetic variants of the vitamin D pathway. While vitamin D supplementation may still play a role in T1D prevention, a large prospective study would be required to substantiate this claim. Overall, our data support recent calls for increased vitamin D supplementation.

CHAPTER 1
INTRODUCTION

Type 1 Diabetes Overview

Type 1 diabetes (T1D), also referred to as juvenile, autoimmune, or insulin-dependent diabetes, is a form of diabetes mellitus that occurs when the body loses its innate ability to produce the insulin hormone necessary for glucose utilization. T1D affects approximately 1 in every 300 people in the United States, and according to the American Diabetes Association, T1D accounts for 5-10% of all diabetes cases. Approximately 85% of newly diagnosed individuals have no prior family history of disease (1). The majority of diagnoses are made in children and young adults, as well as people of European descent (1). The disease process begins with a yet unknown multifactorial immune insult resulting in autoimmune destruction of the insulin-producing beta cells of the pancreatic islets and subsequent inability to maintain euglycemia (2).

The immediate effect of insulin deficiency is a state of hyperglycemia, or elevated glucose in the blood, which can easily progress into a life-threatening condition known as diabetic ketoacidosis (3). When insulin is absent, the body cannot use glucose for energy, so the liver resorts to fat breakdown. This fat breakdown produces ketone bodies that will continue to accumulate and lower the pH of the body to the point of death by acidosis. Inadequate management of blood glucose levels over time can result in a number of complications, including kidney, eye, cardiovascular, and nerve damage.

In addition to the direct health complications associated with T1D, daily life is forever altered due to a routine of constant blood glucose monitoring and insulin dosing in an attempt to achieve euglycemia (4). While enough insulin must be supplied to prevent hyperglycemia, it is just as important to avoid hypoglycemia, or low blood glucose levels, caused by an insulin overdose. Even with the advances in science and technology that have made living with diabetes

more manageable, it is extremely difficult to mimic the body's naturally occurring euglycemic state in the presence of diabetes. Consequently, an enormous effort is under way to both understand the causes of, and develop therapies for, T1D.

T1D has the classical hallmarks of an autoimmune disease, with both environmental and genetic factors playing a role in the disorder's etiopathogenesis (5). Genetically, HLA-DR, HLA-DQ, and HLA-DP allelic variants and the diabetes related autoantibodies GADA, IAA, ICA, and IA2A determine the level of risk an individual may have for developing T1D (6,7). Twin concordance studies have confirmed that genetics are responsible for less than half of one's overall susceptibility to T1D (5), indicative of a powerful environmental influence in T1D etiopathogenesis. Diverse arrays of environmental factors have been associated with the disease, including viruses, infant feeding practices, and childhood immunizations, amongst others (8). Although the exact environmental component remains elusive, a number of published reports suggest vitamin D status may play a role (9, 10, 11).

Vitamin D and Type 1 Diabetes

The link between type 1 diabetes (T1D) and vitamin D emerged with data suggesting that subjects developing the disease had lower serum concentrations of this metabolite than healthy controls (12, 13, 14). Around the world, disease incidence has been shown to exhibit seasonality (15). The north-south gradient hypothesis suggests the number of T1D cases correspond to distance from the equator. In other words, the amount of UV-B exposure determined by geographical location correlates with the frequency of disease. Published reports of greater incidence of disease at higher latitudes support this phenomenon (16).

Vitamin D Metabolite

Vitamin D is a fat-soluble prohormone that regulates calcium and phosphorus levels in the body. Production of the primary source of circulating vitamin D begins via a photochemical

reaction of solar ultraviolet B (UV-B) radiation (wavelength 290-315 nm) with 7-dehydrocholesterol in the skin (17). This multi-step process, as outlined by Holick, continues with cutaneous synthesis of vitamin D3 (cholecalciferol) which undergoes hydroxylation in the liver by 25-hydroxylase enzymes to create the circulating metabolite, known as 25(OH)-vitamin D, or calcidiol (17). Next, calcidiol is hydroxylated in the kidneys by 1α-hydroxylase enzymes to produce the biologically active form of vitamin D, known as 1,25(OH)2-vitamin D, or calcitriol. Calcitriol binds the vitamin D plasma carrier protein, known as vitamin D binding protein (VDBP), which transports the metabolite to various tissues throughout the body. Nuclear vitamin D receptors (nVDRs) are located in nearly all tissues of the body, including antigen-presenting cells and activated T cells of the immune system (17, 18). Consequently, vitamin D regulates, in part, gene expression of cells that possess functional nVDRs.

Many factors may influence UV-B exposure and the ability to synthesize vitamin D, such as cloud cover, smog, geographical location, season, clothing, sunscreen use, melanin, body mass index, age, and diet. Aside from sunlight exposure, dietary vitamin D is available as vitamin D2 (ergocalciferol) and D3 (cholecalciferol), derived from irradiated plant and animal sources respectively. While foods such as butter, milk, and cereal are often fortified with vitamin D, the content is far less than required to meet the recommended daily requirements (18). Vitamin D deficiency leads to the development of rickets and osteomalacia. Vitamin D deficiency has been defined as ≤ 20 ng/mL, insufficiency as 21-30 ng/mL, and sufficiency as > 30 ng/mL (17, 19).

Vitamin D Binding Protein

Vitamin D binding protein (VDBP), also known as Group Specific Component (Gc), is a highly polymorphic plasma protein with a molecular weight of 52-59 kDa (20). The polymorphisms give rise to 3 major isotypes, named Gc2, Gc1s, and Gc1f, which differ by

amino acid substitutions and glycosylation, and their frequencies vary according to geographic location (21). VDBP is synthesized by the liver and released into the blood where it binds and transports vitamin D metabolites, scavenges actin, binds fatty acids, and has a role in macrophage activation and chemotaxis (21). The published normal VDBP plasma concentration range is 300-600 µg/mL (21). Additionally, VDBP concentrations are markedly higher than its vitamin D ligands (22), and no complete absence of VDBP has ever been detected in humans. Its properties suggest it possesses a unique ability to modulate inflammatory immune responses.

Vitamin D Pathway Associated Polymorphisms

Vitamin D polymorphisms, such as the promoter polymorphism CYP27B1 -1260 and the intronic SNP CYP27B1 +2838, have been implicated in T1D (23). Polymorphisms in the CYP27B1 gene are of importance because it encodes the enzyme responsible for converting vitamin D3 into its biologically active form. Polymorphisms in nuclear vitamin D receptors (nVDRs) present in a variety of cells throughout the body may adversely affect receptor binding of vitamin D metabolites, leading to an impaired transcription of genes regulated by vitamin D (18).

Protective Effects of Vitamin D

In a meta-analysis of data from several European studies, children supplemented with vitamin D reduced their risk of developing type 1 diabetes (T1D) by 29% (24). Investigation of T1D animal models revealed that removal of vitamin D accelerates onset of diabetes while pharmacological treatment with vitamin D analogues served to prevent or delay disease (25, 26). This protective effect was also noted among individuals at risk for the autoimmune disease multiple sclerosis who maintained sufficient levels of vitamin D (27). Similar to T1D studies, numerous reports have shown that the incidence of multiple sclerosis decreases with increased UV-B exposure and proximity to the equator (28). Vitamin D may also protect against

autoimmune diseases like T1D that have been linked to viral infection. Specifically, vitamin D induces cathelicidin, an antimicrobial peptide, increasing the efficacy of fighting infections, thereby reducing risk of disease progression (29).

Immunomodulatory Role of Vitamin D

The presence of nuclear vitamin D receptors (nVDRs) in immune system cells suggests a possible role for vitamin D in regulating the immune response. Activated macrophages and dendritic cells have been shown to contain the enzyme responsible for converting vitamin D into its biologically active form, calcitriol (18). Calcitriol has been shown to promote phagocytosis by macrophages (18) as well as the development of Th2 lymphocytes (30). Additionally, calcitriol is capable of downregulating antigen presentation and production of inflammatory cytokines like IL-2 and IL-12 (30). Vitamin D exerts its effects on hundreds of genes, ultimately influencing cell proliferation, differentiation, and death (17).

Introduction to Experimental Design

Numerous studies, the majority of which were based out of northern European countries, have reported lower vitamin D serum concentrations in patients with type 1 diabetes (T1D) compared to healthy controls. One study performed in the United States, also suggesting reduced vitamin D levels in T1D patients, failed to provide values amongst healthy control individuals and hence, did not identify disease specificity. In an attempt to contribute to this model and fill an underlying knowledge void, we addressed the basis for this association in a solar-rich environment. Specifically, we sought to measure serum 25(OH)-vitamin D levels from T1D patients, first-degree relatives of diabetic patients, and healthy controls resident to Florida, USA.

Additional support for a role of vitamin D in the autoimmune process of T1D comes from studies that have shown an association between specific vitamin D binding protein (VDBP)

polymorphisms and T1D markers (31). Genetic variants of VDBP are of significance because they alter the binding affinity of the carrier protein for vitamin D. While genotypic VDBP associations have been observed, phenotypic associations have not been made. For example, serum levels of VDBP have neither been measured nor compared among diabetics and nondiabetics. For this reason, we sought to measure VDBP levels from T1D patients, their first-degree relatives, and healthy controls.

CHAPTER 2
MATERIALS AND METHODS

Serum 25(OH)-Vitamin D Levels

We obtained serum from 415 individuals in Florida, divided into the following cohorts: controls (median age 22.0 years; range 5-65 years; females =84, total =153), subjects with new-onset T1D, defined as ≤ 5 months duration (median age 12.2; range 5.9-35.0; females =23; total =46), those with established disease in which presence of T1D extended beyond 5 months (median age 16.0; range 5.1-62.6; females =50; total =110), and relatives of those with T1D (median age 21.0; range 1.0-62.6; females =54; total =106). All samples were collected under informed consent with University of Florida Institutional Review Board approval.

25(OH)-vitamin D levels were quantified in duplicate with a commercial EIA kit (ALPCO; Salem, NH) using 50µL of banked serum from each subject; an analyte shown previously as stable under such conditions (32). The levels of 25(OH)-vitamin D were interpolated from a standard curve after reading the absorbance on a M5 Spectramax plate reader using Softmax Pro 4.8 software (Molecular Devices, Sunnyvale, CA). The intra- and inter-assay coefficients of variation for this assay were 10.7% and 13.2% respectively. 25(OH)-vitamin D deficiency was defined as less than or equal to 20 ng/mL, insufficiency as 21-30 ng/mL, and sufficiency as > 30 ng/mL (17,19).

UV Index (UVI) climatological data was obtained from the National Weather Service (NWS) and United States Environmental Protection Agency (EPA) websites (http://www.cpc.ncep.noaa.gov and http://www.epa.gov) to determine relative UV exposure. Based on the previous five years worth of data for the proximate city of Jacksonville, FL, we established UV exposure monthly means: January: 3.215, February: 4.08, March: 5.96, April: 7.68, May: 8.238, June: 8.578, July: 8.976, August: 8.254, September: 6.902, October: 5.11,

November: 3.694, and December: 2.79. The numbers correspond to the UVI scale (1-11+) developed by the NWS and EPA and implemented by the World Health Organization (WHO).

Vitamin D Binding Protein Levels

We obtained serum from 472 individuals, 386 of which we had previously measured 25(OH)-vitamin D levels on. The samples included 153 controls, 203 T1D subjects (new-onset and established), and 116 first-degree relatives of T1D subjects. All samples were from individuals resident to Florida, USA and collected under informed consent with University of Florida Institutional Review Board approval.

VDBP levels were quantified in duplicate with a commercial EIA kit (ALPCO; Salem, NH) using 10µL of banked serum from each subject. The levels of VDBP were interpolated from a standard curve after reading the absorbance on a M5 Spectramax plate reader using Softmax Pro 4.8 software (Molecular Devices, Sunnyvale, CA). The intra- and inter-assay coefficients of variation for this assay were 5.0% and 12.7% respectively. The normal reference range for VDBP levels was 300-600 µg/mL or 30-60 mg/dL (21).

Statistical Analysis

For 25(OH)-vitamin D data, analysis of multiple, unpaired group comparisons was achieved using the non-parametric Kruskal-Wallis test with Dunn's post-test. The relationship between age and 25(OH)-vitamin D levels was analyzed by linear regression. All analyses were done using GraphPad Prism software ver 5.00 (H.J. Motulsky, Analyzing Data with GraphPadPrism, 1999, GraphPad Software Inc., San Diego, CA, www.graphpad.com).

For VDBP data, analysis of multiple, unpaired group comparisons was achieved using the non-parametric Kruskal-Wallis test with Dunn's post-test. The relationship between 25(OH)-vitamin D levels and VDBP levels was analyzed by linear regression. Additionally, the association of age and disease duration with VDBP levels was considered using linear regression

analysis. To determine the relationship between VDBP levels and gender, the non-parametric Mann-Whitney test was used.

CHAPTER 3
RESULTS

Serum 25(OH)-Vitamin D

25(OH)-vitamin D levels (mean ng/mL; 95% CI) were as follows: healthy controls (27.2; 23.3-31.1), new-onset T1D (21.8; 18.2-25.3), established T1D (27.7; 22.4-33.1), and first-degree relatives (23.7; 21.0-26.3) (Figure 3-1). The medians did not vary significantly among individuals at varying degrees of disease risk ($p = 0.705$). Suboptimal vitamin D levels (≤ 30 ng/mL) were observed in 70.1% of controls, 76.1% of new-onset T1D subjects, 68.5% of patients with established T1D, and 68.8% of relatives; values that while low, were not significantly different from each other (Table 3-1; p =NS).

Next, previous reports have stated that vitamin D deficiency is most prevalent among children, adolescents, and the elderly and that lower vitamin D levels are found in men versus women (33, 34). As such, we compared gender-segregated (Figure 3-2) and age-segregated (Figure 3-3) 25(OH)-vitamin D levels. No significant gender differences were observed ($p = 0.493$). Using linear regression, we compared age and serum 25(OH)-vitamin D levels in our overall study population and the individual cohorts. For all groups combined, $r^2 = 0.004$ $p = 0.224$; healthy controls, $r^2 = 0.010$ $p = 0.214$; new-onset T1D, $r^2 = 0.0001$ $p = 0.963$; established T1D, $r^2 = 0.013$ $p = 0.238$; and relatives, $r^2 = 0.075$ $p = 0.005$. In sum, regression analysis revealed no trend in vitamin D levels as it pertained to age overall (p =NS) but the relatives subgroup did reveal a significant inverse relationship with age ($p = 0.005$, data not shown).

Since sunlight plays a major role in vitamin D synthesis, we then examined vitamin D levels as a function of the month the sample was drawn, and therefore examined the influence of UV-B exposure. The samples were grouped according to month drawn, and placed into one of four possible 3-month blocks, each block formed on the basis of similar UV-B indices (Figure 3-

4). The 25(OH)-vitamin D (reported as mean ng/mL; 95% CI) levels for the November/December/January group of 112 samples (26.9; 21.6-32.2) had an average estimated UV exposure of 3.23. The October/February/March group of 113 samples (24.2; 20.6-27.9) had an average estimated UV exposure of 5.05. The September/April/May group of 84 samples (26.6; 21.5-31.7) had an average estimated UV exposure of 7.61. Finally, the June/July/August group of 106 samples (25.7; 22.6-28.9) had an average estimated UV exposure of 8.60. Comparison of the vitamin D levels between each three-month block showed no significant difference ($p = 0.779$). Further analysis revealed no significant differences on a monthly exposure basis or examining controls versus new-onset T1D, established T1D, or first-degree relatives (data not shown).

Vitamin D Binding Protein

VDBP concentrations were determined for 3 experimental cohorts: 153 healthy controls, 203 T1D subjects, and 116 relatives of T1D subjects (Figure 3-5). VDBP levels (mean µg/mL; 95% CI) were as follows: healthy controls (528.2; 467.3-589.0), T1D subjects (424.8; 403.6-446.0), and relatives (496.9; 410.3-583.4). Using the non-parametric Kruskal-Wallis with Dunn's post-test, we were able to determine T1D subjects had significantly lower VDBP levels when compared to healthy controls ($p = 0.0028$).

VDBP levels were also compared with 25(OH)-vitamin D levels for 152 controls, 43 new-onset T1D subjects, 98 established T1D subjects, and 93 first-degree relatives. Linear regression analysis of each group is as follows: controls ($p = 0.9194$; $r^2 = 0.00007$) (Figure 3-6), new-onset T1D ($p = 0.2569$; $r^2 = 0.03124$) (Figure 3-7), established T1D ($p = 0.9289$; $r^2 = 0.00008$) (Figure 3-8), and relatives ($p = 0.1459$; $r^2 = 0.02310$) (Figure 3-9). Overall, no significant association was detected among any study population (p=NS).

Without knowledge of the differences that may exist in VDBP levels according to gender, we analyzed VDBP levels of 238 female and 233 male samples using the non-parametric Mann-Whitney method (Figure 3-10). VDBP levels (mean µg/mL; 95% CI) were as follows: females (514.9; 460.8-569.0), and males (435.6; 408.9-462.3). Analysis revealed the means between the two groups were significantly different ($p < 0.0001$).

In an effort to determine the effect of disease duration on the VDBP levels of T1D subjects, we performed linear regression analysis (data not shown) and determined the duration of disease did not significantly impact VDBP levels ($p = 0.5158$; $r^2 = 0.0026$). Along these same lines, we sought to identify whether or not age affected VDBP levels (Figure 3-11). Of 458 samples analyzed by linear regression, no significance was found ($p = 0.1643$; $r^2 = 0.004238$).

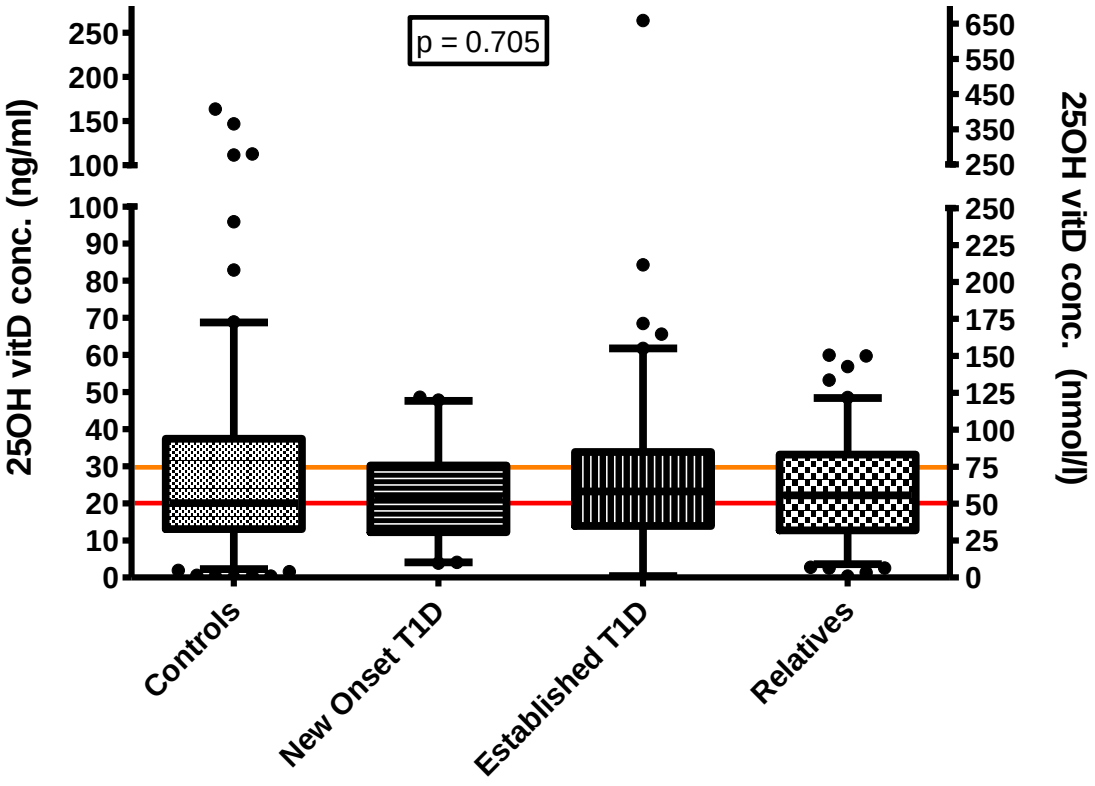

Figure 3-1. Serum 25(OH)-vitamin D levels among four subgroups of our study population (n = 415; p= 0.705). Box and whiskers plot (median, whiskers 5-95 percentile, outliers •). Orange and red lines represent the cutoffs for insufficiency and deficiency respectively. Left y-axis, 25(OH)-vitamin D ng/mL; right y-axis, 25(OH)-vitamin D nmol/L.

Table 3-1. Classification of vitamin D status in relation to type 1 diabetes risk

	Normal > 30ng/mL N (%)	Insufficient 20-30ng/mL N (%)	Deficient < 20ng/mL N (%)
Healthy Controls (n=153)	46 (30.1)	31 (20.3)	76 (49.7)
New Onset T1D (n=46)	11 (23.9)	13 (28.3)	22 (47.8)
Established T1D (n=110)	34 (30.9)	29 (26.4)	47 (42.7)
Relatives (n=106)	34 (32.1)	22 (20.8)	50 (47.2)

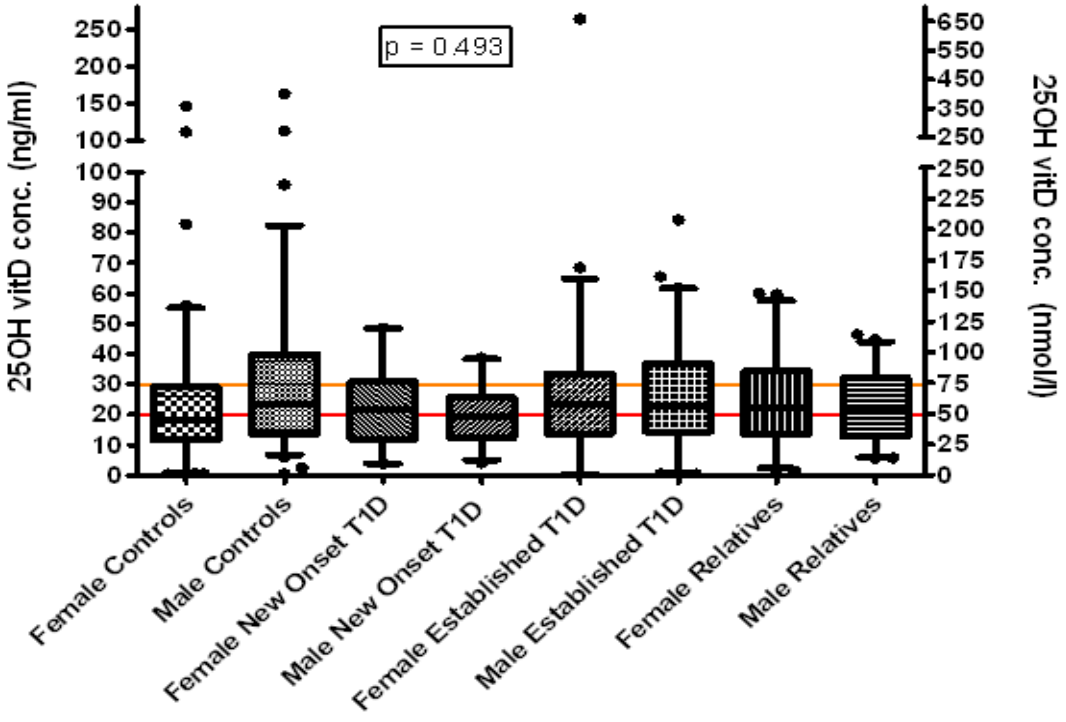

Figure 3-2. Female and male serum 25(OH)-vitamin D levels in each experimental cohort (p =0.493). Box and whiskers plot (median, whiskers 5-95 percentile, outliers •). Orange and red lines represent the cutoffs for insufficiency and deficiency respectively. Left y-axis, 25(OH)-vitamin D ng/mL; right y-axis, 25(OH)-vitamin D nmol/L.

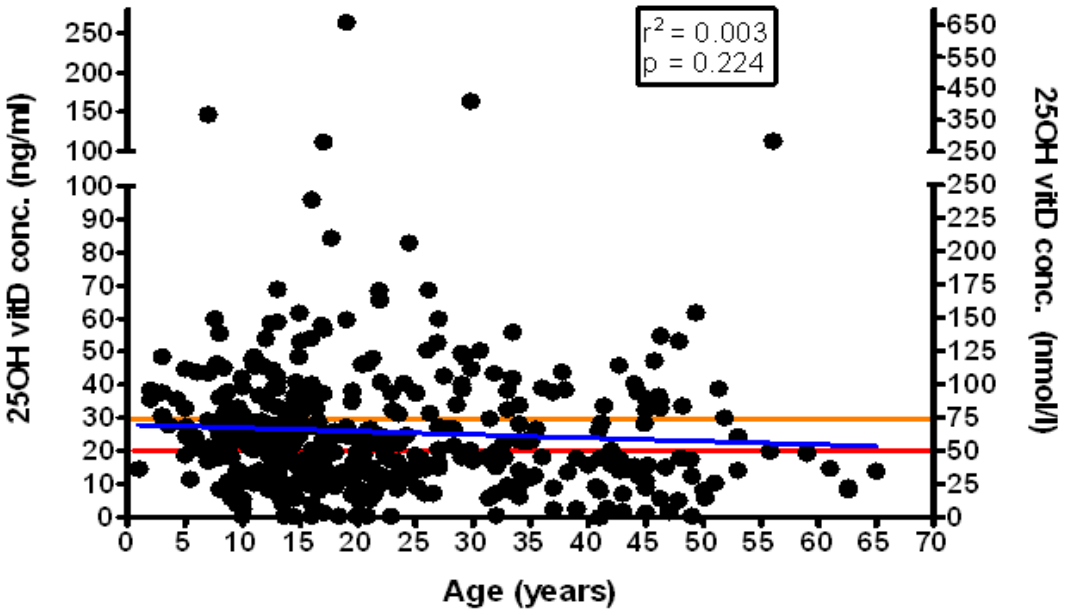

Figure 3-3. Age vs. serum 25(OH)-vitamin D levels. Orange and red lines represent the cutoffs for 25(OH)-vitamin D insufficiency and deficiency respectively. Blue line indicates linear regression ($r^2 = 0.003$; $p = 0.224$).

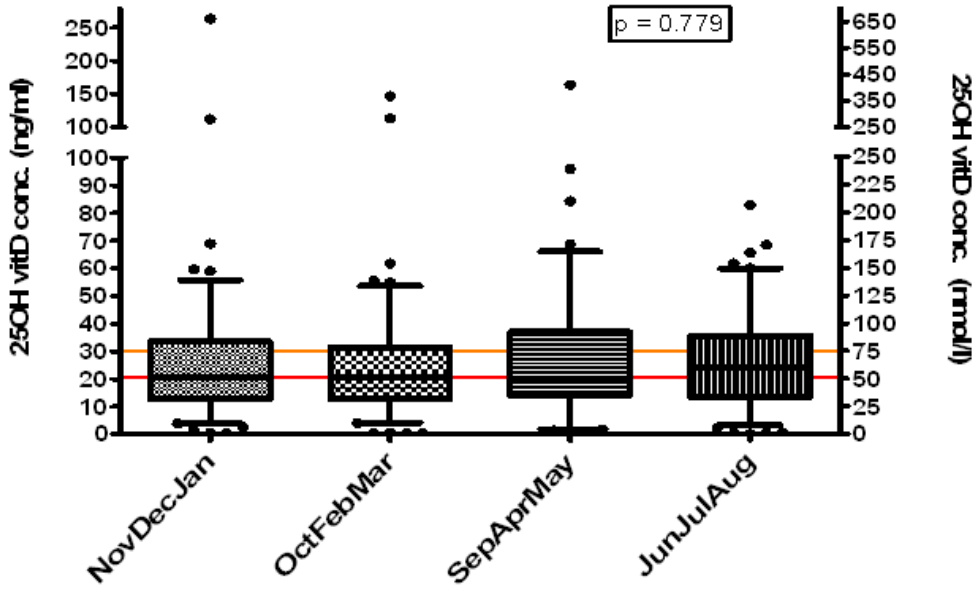

Figure 3-4. Impact of estimated UV-B exposure on serum 25(OH)-vitamin D levels. Orange and red lines represent the cutoffs for 25(OH)-vitamin D insufficiency and deficiency respectively. Samples categorized by 3 month blocks with the most similar estimated UV exposure rates ($p = 0.779$).

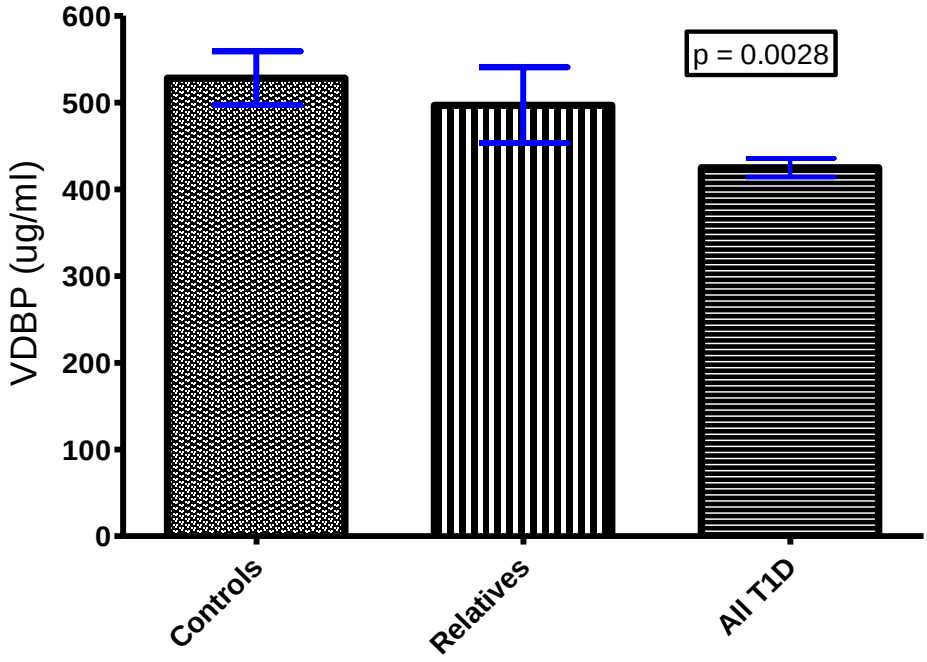

Figure 3-5. Vitamin D binding protein levels (µg/mL) among 3 subgroups of our study population (n = 472; p = 0.0028). Bar graph; blue lines represent mean with SEM.

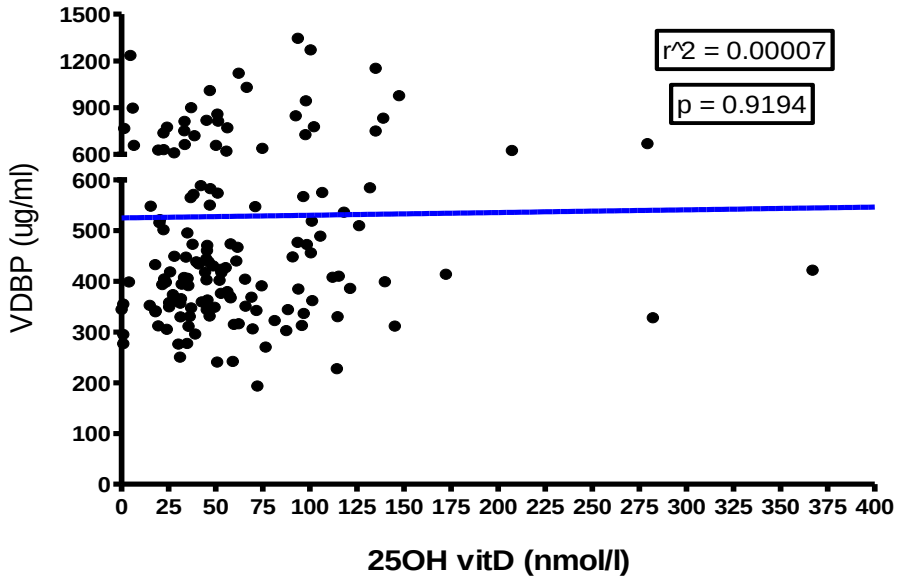

Figure 3-6. Serum 25(OH)-vitamin D levels (nmol/L) vs. vitamin D binding protein levels (µg/mL) in healthy controls. Blue line indicates linear regression (r^2 = 0.00007; p = 0.9194).

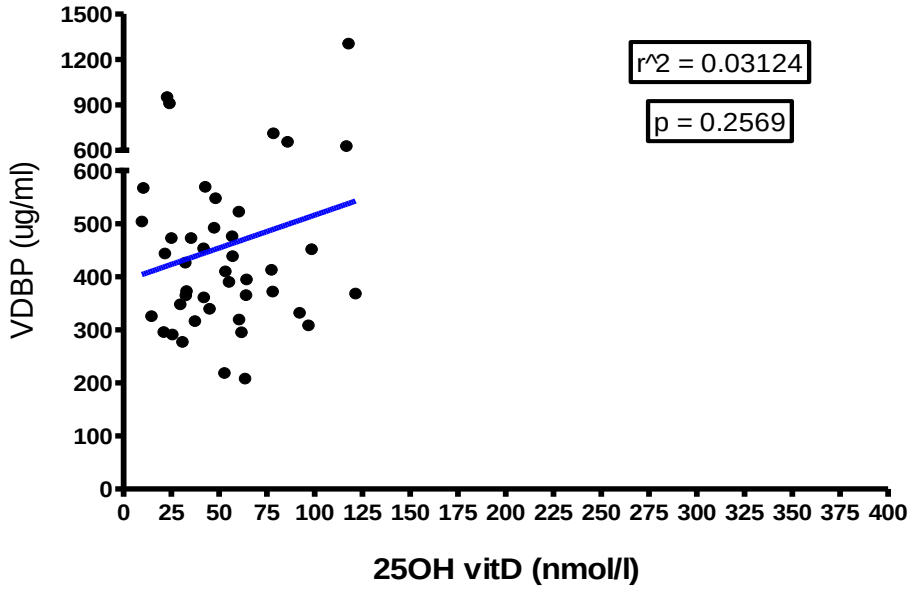

Figure 3-7. Serum 25(OH)-vitamin D levels (nmol/L) vs. vitamin D binding protein levels (µg/mL) in subjects with new-onset type 1 diabetes. Blue line indicates linear regression (r^2 = 0.03214; p = 0.2569).

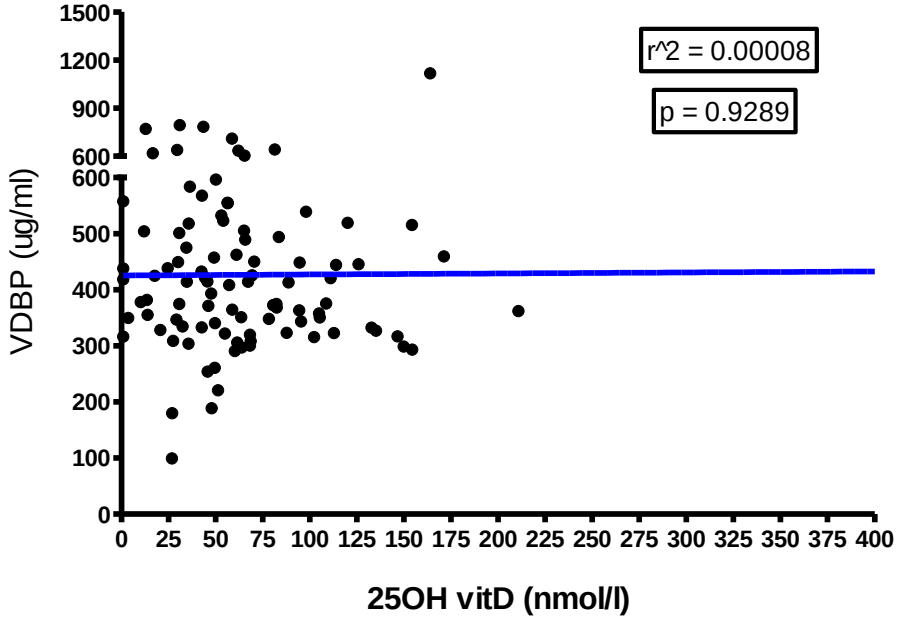

Figure 3-8. Serum 25(OH)-vitamin D levels (nmol/L) vs. vitamin D binding protein levels (µg/mL) in subjects with established type 1 diabetes. Blue line indicates linear regression (r^2 = 0.00008; p = 0.9289).

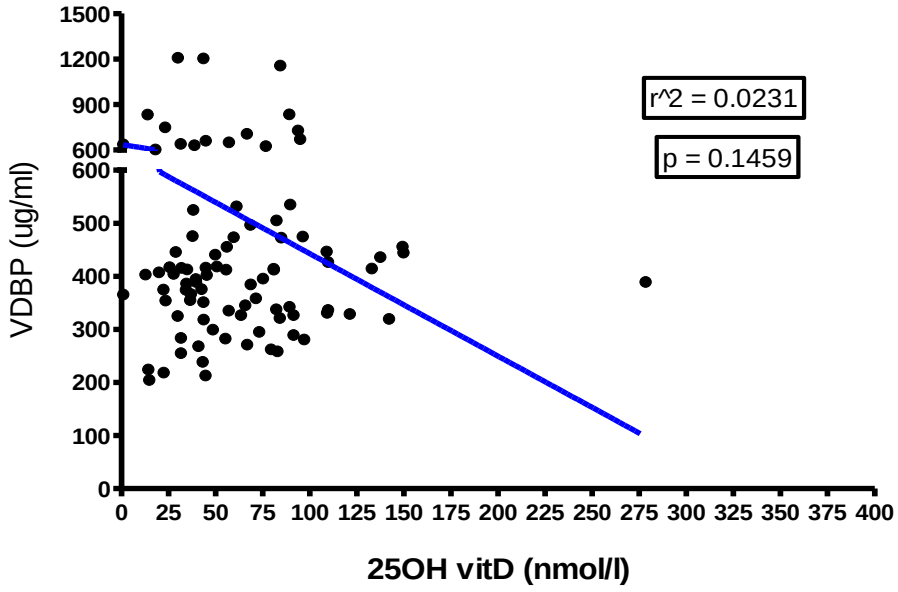

Figure 3-9. Serum 25(OH)-vitamin D levels (nmol/L) vs. vitamin D binding protein levels (µg/mL) in first-degree relatives of type 1 diabetics. Blue line indicates linear regression ($r^2 = 0.0231$; $p = 0.1459$).

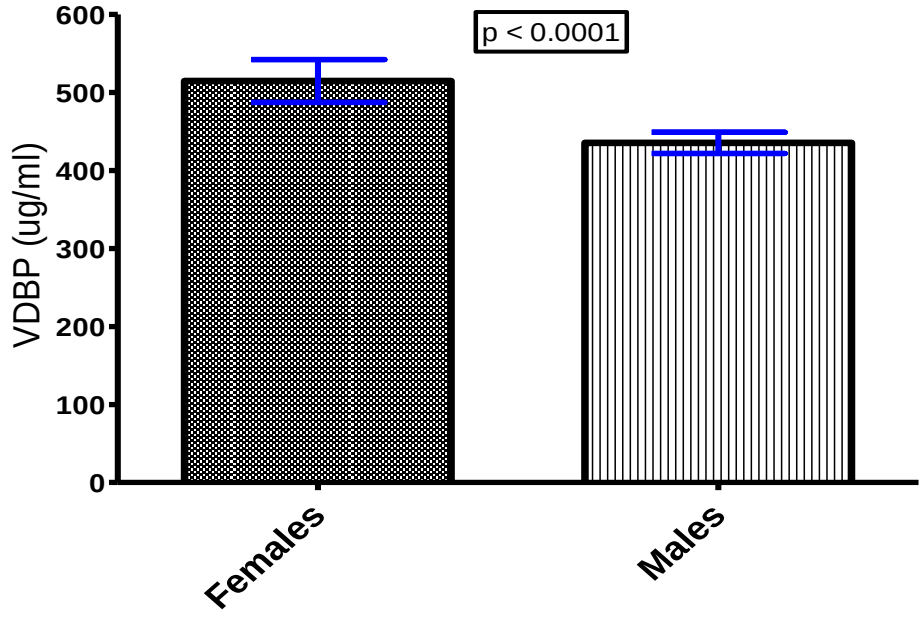

Figure 3-10. Vitamin D binding protein levels (µg/mL) in female (n = 238) and male (n = 233) groups ($p < 0.0001$). Bar graph; blue lines represent mean with SEM.

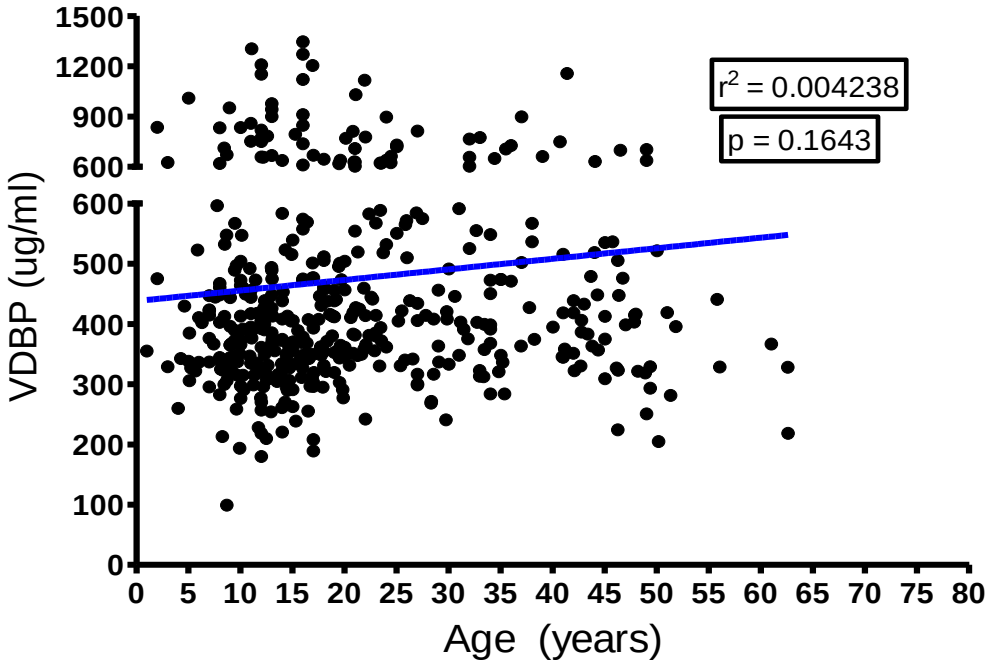

Figure 3-11. Age vs. vitamin D binding protein levels (µg/mL). Blue line indicates linear regression (r2 = 0.004238; p = 0.1643).

CHAPTER 4
DISCUSSION

Serum 25(OH)-Vitamin D Levels in Type 1 Diabetes

Our study did not find significant differences in vitamin D levels between healthy controls, subjects with T1D, and first-degree relatives, using samples obtained in a solar rich region of the United States. However, to our surprise, we identified that within each group there exists a high frequency of vitamin D insufficiency despite the sun rich environment of Florida. Specifically, vitamin D levels in more than two-thirds of each population were below the recommended 30 ng/mL level; placing these subjects in the category of vitamin D insufficient or deficient.

While we may have an ecological fallacy bias in assigning UV Index aggregate data to individual subjects, other factors such as sun avoidance practices to inadequate supplementation may also account for the low 25(OH)-vitamin D levels observed in this cross sectional study. Additionally, at Florida's latitude, the duration of sunlight hours/day does not vary as dramatically as seen as one approaches the poles. Florida's geographical location allows for cutaneous synthesis of vitamin D year-round.

Given the amount of UV available to Florida residents and the fortification of milk products with vitamin D, the low serum levels of vitamin D found add credence to the recent recommendation by the American Academy of Pediatrics to double the amount of vitamin D supplementation provided to children (35). Worth noting are recent studies that suggest vitamin D3 is more efficient at raising serum 25(OH)-vitamin D levels than D2 (36). This indicates that supplementing with vitamin D2 alone may prove ineffective at raising serum 25(OH)-vitamin D to an optimal level. Overall, vitamin D is an essential prohormone not only necessary for the usual role in calcium homeostasis, but also vital for immune function.

Vitamin D Binding Protein Levels in Type 1 Diabetes

Despite a lack of association of 25(OH)-vitamin D levels with healthy controls, T1D subjects, and first-degree relatives of T1D subjects, we did find a significant difference in the VDBP levels when comparing control and T1D study populations. Interestingly, mean VDBP levels were highest in controls and lowest in T1D subjects, with VDBP levels of first-degree relatives at an intermediate level. The VDBP concentration mean for each group fell within the normal VDBP concentration range of 300-600 µg/mL, however, there were a number of values in each study population that fell outside this reference range. This may be explained by the daily fluctuations that occur with VDBP concentrations. VDBP levels undergo a decline in the morning, followed by a rapid increase, and finally, a plateau throughout the remainder of the day (20).

Investigation into the relationship between 25(OH)-vitamin D levels and VDBP levels of healthy controls, new-onset T1D subjects, established T1D subjects, and first-degree relatives of T1D subjects failed to reveal any significant association. This was not too surprising since VDBP concentrations are not regulated by vitamin D metabolites, and because VDBP circulates at a much higher concentration than its ligand (22). However, a recent study of healthy women that measured the serum levels of 1,25(OH)2-vitamin D, the active vitamin D metabolite, found a positive correlation with VDBP concentrations (37).

After further analysis, we did not find a significant relationship between duration of T1D and VDBP levels or between age and VDBP levels. However, we discovered that VDBP levels were significantly higher in women compared to men, although the VDBP means for females and males were maintained within the normal reference range of 300-600 µg/mL. This association warrants further investigation.

Conclusion

The use of vitamin D analogues for delaying or preventing the development of type 1 diabetes (T1D) may have potential, but will require additional research to develop a regimen that is safe and timely, as well as applicable to all individuals at an increased risk genetically for developing T1D. Our data supports the need for increasing the current recommendations set for vitamin D supplementation. This recommendation is based on finding suboptimal vitamin D levels in the majority of our entire study population despite an environment with abundant sunlight. The presence of significantly lower levels of vitamin D binding protein (VDBP) in T1D subjects further supports the notion that the vitamin D pathway may have a role in disease pathogenesis. Also worth considering is the impact low vitamin D levels have on the body's ability to fight infection. If sufficient vitamin D levels can be maintained, perhaps the current increasing trends in autoimmune diseases like T1D can be reversed, or at the very least, limited until a cure can be found.

CHAPTER 5
FUTURE RESEARCH DIRECTIONS

In light of our findings of low circulating serum 25(OH)-vitamin D throughout the entire study population in similar proportion, yet significantly lower concentrations of vitamin D binding protein (VDBP) in subjects with type 1 diabetes (T1D), additional studies will be required to determine a definitive role. A large prospective study to further define the contribution of vitamin D in pathogenesis of T1D is urgently needed as trials using the active form of vitamin D for T1D prevention are undergoing discussion. Another priority includes identifying whether or not the differences we observed in VDBP levels correlate with VDBP allelic frequencies of Gc1f, Gc1s, and Gc2 as other reports have shown (38,39). Also of interest is the CYP27B1 gene, which encodes the 1α-hydroxylase enzyme responsible for converting 25(OH)-vitamin D into its bioactive form. Previous studies have shown specific CYP27B1 polymorphisms associate with T1D (10), and we seek to determine whether or not these polymorphisms associate with reduced 1α-hydroxylase, 25(OH)-vitamin D, and VDBP levels.

LIST OF REFERENCES

1. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet*. 2001; 358 (9277): 221-229.

2. Morran MP, McInerney MF, Pietropaolo M. Innate and adaptive autoimmunity in type 1 diabetes. *Pediatr Diabetes*. 2008; 9(2): 152-161.

3. Kitabchi AE, Wall BM. Management of diabetic ketoacidosis. *Am Fam Physician*. 1999; 60(2): 455-464.

4. Scheidegger U, Allemann S, Scheidegger K, et al. Continuous subcutaneous insulin infusion therapy: effects on quality of life. *Swiss Med Wkly*. 2007; 137(33-34): 476-482.

5. Kim MS, Polychronakos C. Immunogenetics of type 1 diabetes. *Horm Res*. 2005; 64(4): 180-188.

6. Alizadeh BZ, Koeleman BP. Genetic polymorphisms in susceptibility to type 1 diabetes. *Clin Chim Acta*. 2008; 387(1-2): 9-17.

7. Mrena S, Virtanen SM, Laippala P, et al. Models for predicting type 1 diabetes in siblings of affected children. *Diabetes Care*. 2006; 29(3): 662-667.

8. Gale EA. Spring harvest? Reflections on the rise of type 1 diabetes. *Diabetologia*. 2005; 48(12): 2445-2450.

9. Peechakara SV, Pittas AG. Vitamin D as a potential modifier of diabetes risk. *Nat Clin Pract Endocrinol Metab*. 2008; 4(4): 182-183.

10. Bailey R, Cooper JD, Zeitels L, et al. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. Diabetes. 2007; 56(10): 2616-2621.

11. Ramos-Lopez E, Jansen T, Ivaskevicius V, et al. Protection from type 1 diabetes by vitamin D receptor haplotypes. *Ann N Y Acad Sci*. 2006; 1079: 327-334.

12. Pozzilli P, Manfrini S, Crino A, et al. Low levels of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. *Horm Metab Res*. 2005; 37(11): 680-683.

13. Littorin B, Blom P, Scholin A, et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Stud in Sweden (DISS). *Diabetologia*. 2006; 49(12): 2847-2852.

14. Svoren BM, Volkening LK, Wood JR, et al. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. *J Pediatr*. 2009; 154(1): 132-134.

15. Mooney JA, Helms PJ, Jolliffe IT, et al. Seasonality of type 1 diabetes mellitus in children and its modifications by weekends and holidays: retrospective observational study. *Arch Dis Child*. 2004; 89: 970-973.

16. Mohr SB, Garland CF, Gorham ED, et al. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia*. 2008; 51(8): 1391-1398.

17. Holick, MF. Vitamin D deficiency. *N Engl J Med*. 2007; 357(3): 266-281.

18. Mathieu C, Gysemans C, Giulietti A, et al. Vitamin D and diabetes. *Diabetologia*. 2005; 48(7): 1247-1257.

19. Holick MF. Diabetes and the vitamin D connection. *Curr Diab Rep*. 2008; 8(5): 393-398.

20. Speeckaert M, Huang G, Delanghe JR, et al. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin Chim Acta*. 2006; 372(1-2): 33-42.

21. Gomme PT, Bertolini J. Therapeutic potential of vitamin D-binding protein. *Trends Biotechnol*. 2004; 22(7): 340-345.

22. Cooke NE, Haddad JG. Vitamin D binding protein (Gc-globulin). *Endocr Rev*. 1989; 10(3): 294-307.

23. Bailey R, Cooper JD, Zeitels L, et al. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes*. 2007; 56(10): 2616-2612.

24. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child*. 2008; 93(6): 512-517.

25. Giulietti A, Gysemans C, Stoffels K, et al. Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. *Diabetologia*. 2004; 47(3): 451-462.

26. Giarratana N, Penna G, Amuchastequi S, et al. A vitamin D analog down-regulates proinflammatory chemokine production by pancreatic islets inhibiting T cell recruitment and type 1 diabetes development. *J Immunol*. 2004; 173(4): 2280-2287.

27. Munger KL, Levin LI, Hollis BW, et al. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. *JAMA*. 2006; 296(23): 2832-2838.

28. Raghuwanshi A, Joshi SS, Christakos S. Vitamin D and multiple sclerosis. *J Cell Biochem*. 2008; 105(2): 338-343.

29. Grant WB. Hypothesis-ultraviolet-B irradiance and vitamin D reduce the risk of viral infections and their sequelae, including autoimmune diseases and some cancers. *Photochem Photobiol*. 2008; 84(2): 356-365.

30. Alpert PT, Shaikh U. The effects of vitamin D deficiency and insufficiency on the endocrine and paracrine systems. *Biol Res Nurs*. 2007; 9(2): 117-129.

31. Onganga JC, Pinget M, Belcourt A. Vitamin D-binding protein gene polymorphism association with IA-2 autoantibodies in type 1 diabetes. *Clin Biochem*. 2005; 38(5): 415-419.

32. Bodnar LM, Catov JM, Wisner KL, et al. Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. *Br J Nutr*. 2009; 101(2): 278-284.

33. Holick MF. The influence of vitamin D on bone health across the life cycle. *J Nutr*. 2005; 135(11): 2726-2727.

34. Hagenau T, Vest R, Gissel TN, et al. Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. *Osteoporos Int*. 2009; 20(1): 133-140.

35. Wagner CL, Greer FR. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008; 122(5): 1142-1152.

36. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr*. 2006; 84(4): 695-697.

37. Bouillon R, Van Assche FA, Van Baelen H, et al. Influence of the vitamin D-binding protein on the serum concentration of 1,25-dihhydroxyvitamin D3. Significance of the free 1,25-dihydroxyvitamin D3 concentration. *J Clin Invest*. 1981; 67(3): 589-596.

38. Lauridsen AL, Vestergaard P, Heickendorff L, et al. Plasma concentrations of 25-hydroxyvitamin D and 1,25-hydroxyvitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int*. 2005; 77(1): 15-22.

39. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc-globulin) is related to the Gc phenotype in women. *Clin Chem*. 2001; 47(4): 753-756.

BIOGRAPHICAL SKETCH

Lindsey Marie Bierschenk was born in St. Louis, Missouri, where she attended Francis Howell High School for grades 9–12. In her senior year of high school, she was diagnosed with type 1 diabetes. She received a Bachelor of Science degree from the University of Missouri–Columbia in 2007. Her major was biology with a minor in Spanish. As an undergraduate, Lindsey studied approaches for delaying onset of type 1 diabetes in the NOD mouse model. In 2007, she joined the laboratory of Dr. Mark Atkinson where she studied the role of vitamin D in individuals with type 1 diabetes. In May of 2009, she received a Master of Science degree in Medical Sciences from the University of Florida.