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A Cross-Sectional Study of Vitamin D, Glycemic Control, and Inflammatory Cytokines in Children and Adolescents With Type 1 Diabetes Mellitus

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Abstract

Background: Vitamin D deficiency is markedly prevalent in children and adolescents with type 1 diabetes mellitus (T1DM). Despite accumulating evidence to support the link between vitamin D deficiency and both impaired glucose metabolism and altered immune responses *in vitro* and *in vivo*, it is not known whether vitamin D deficiency is linked to poor glycemic control and/or systemic levels of inflammatory cytokines in children and adolescents with T1DM. **Aims**: The purpose of this study was to examine the relationship between 25-hydroxyvitamin D (the functional indicator of vitamin D status) and HbA1c (the standardized index of glycemic control); and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. **Methods**: A cross-sectional design was used to examine these relationships in a convenience sample of 197 children and adolescents with T1DM 7-18 years, recruited from the Diabetes Center for Children at the Children's Hospital of Philadelphia. Non-fasting serum levels of 25-hydroxyvitamin D, IL-6, IL-8, IL-10, and blood glucose were measured. Data on socio-demographic and disease-related variables including HbA1c were abstracted from medical records. Age- and sex-specific body mass index standard deviation scores (BMI z-score) were calculated. General linear modeling was used to examine the hypothesized relationships between primary variables, while controlling for select socio-demographic and disease-related covariates. **Results**: Mean HbA1c was 8.6±1.4%; 22.8% subjects had poor glycemic control. Mean 25-hydroxyvitamin D was 54.6±17.8nmol/L; 90.0% subjects had 25-hydroxyvitamin D levels less than 75nmol/L. Mean serum IL-6 was highest in overweight/obese adolescent females (1.57±1.29pg/ml). Mean serum IL-10 was highest in African Americans with poor glycemic control (15.2±22.5pg/ml). Serum levels of IL-8 were not detected in this sample. 25-hydroxyvitamin D was not associated with HbA1c (β=0.008; P=0.108), nor with IL-6 (β=-0.005; P=0.175) or IL-10 (β=0.004; P=0.356). IL-6 was not associated with HbA1c (β=0.09; P=0.418). IL-10 was significantly associated with HbA1c (β=0.21; P=0.008). **Conclusions:** In this study sample of metabolically stable children and adolescents with T1DM, neither 25-hydroxyvitamin D nor IL-6 were significantly associated with HbA1c. IL-10 was significantly associated with HbA1c. Clinical controlled trials are needed to confirm these results and assess the effect of varied doses of vitamin D supplements on inflammatory cytokines and ultimately measure the effect of inflammatory cytokines on HbA1c in T1DM.

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A CROSS-SECTIONAL STUDY OF VITAMIN D, GLYCEMIC CONTROL, AND INFLAMMATORY CYTOKINES IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

Sarah Ibrahim Al Sawah

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DEDICATION

I dedicate this work to my family. Words are not enough to express my gratitude to my parents, Ibrahim and Kamleh Al Sawah whose love, words of encouragement and prayers have helped me stay focused and achieve this great milestone in my life. My brothers Samuel and Timothy have been great role models through their achievements, motivation, ambition and tenacity.

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ABSTRACT

A CROSS-SECTIONAL STUDY OF VITAMIN D, GLYCEMIC CONTROL, AND INFLAMMATORY CYTOKINES IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES MELLITUS

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Background: Vitamin D deficiency is markedly prevalent in children and adolescents with type 1 diabetes mellitus (T1DM). Despite accumulating evidence to support the link between vitamin D deficiency and both impaired glucose metabolism and altered immune responses *in vitro* and *in vivo*, it is not known whether vitamin D deficiency is linked to poor glycemic control and/or systemic levels of inflammatory cytokines in children and adolescents with T1DM. **Aims**: The purpose of this study was to examine the relationship between 25-hydroxyvitamin D (the functional indicator of vitamin D status) and HbA1c (the standardized index of glycemic control); and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. **Methods**: A cross-sectional design was used to examine these relationships in a convenience sample of 197 children and adolescents with T1DM 7-18 years, recruited from the Diabetes Center for Children at the Children's Hospital of Philadelphia. Non-fasting serum levels of 25-hydroxyvitamin D, IL-6, IL-8, IL-10, and blood glucose were measured. Data on socio-demographic and disease-related variables including HbA1c were abstracted from medical records. Age- and sex-specific body mass index standard deviation scores (BMI z-score) were calculated. General linear modeling was used to examine the hypothesized relationships between primary variables, while controlling for select socio-demographic and disease-related covariates. **Results**: Mean HbA1c was 8.6±1.4%; 22.8% subjects had poor glycemic control. Mean 25-hydroxyvitamin D was 54.6±17.8nmol/L; 90.0% subjects had 25-hydroxyvitamin D levels less than 75nmol/L. Mean serum IL-6 was highest in

overweight/obese adolescent females (1.57±1.29pg/ml). Mean serum IL-10 was highest in African Americans with poor glycemic control (15.2±22.5pg/ml). Serum levels of IL-8 were not detected in this sample. 25-hydroxyvitamin D was not associated with HbA1c (β=0.008; P=0.108), nor with IL-6 (β=-0.005; P=0.175) or IL-10 (β=0.004; P=0.356). IL-6 was not associated with HbA1c (β=0.09; P=0.418). IL-10 was significantly associated with HbA1c (β=0.21; P=0.008). **Conclusions:** In this study sample of metabolically stable children and adolescents with T1DM, neither 25-hydroxyvitamin D nor IL-6 were significantly associated with HbA1c. IL-10 was significantly associated with HbA1c. Clinical controlled trials are needed to confirm these results and assess the effect of varied doses of vitamin D supplements on inflammatory cytokines and ultimately measure the effect of inflammatory cytokines on HbA1c in T1DM.

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CHAPTER ONE: INTRODUCTION

Type 1 diabetes mellitus (T1DM) is the third most prevalent chronic disease of childhood, affecting one in every 400 to 600 children and adolescents in the United States (SEARCH for Diabetes in Youth Study Group et al., 2006; Centers for Disease Control and Prevention [CDC], 2008). The Philadelphia Pediatric Diabetes Registry showed that the age-adjusted incidence rate of T1DM in children 0-14 years was 18.2 per 100,000/year in White children, 14.0 per 100,000/year in Black children, and 14.0 per 100,000/year in Hispanic children from 2000 to 2004 (Lipman et al., 2009). Children and adolescents with T1DM are at increased risk to develop micro- and macro-vascular complications such as nephropathy, neuropathy, retinopathy, and coronary and peripheral vascular disease later in life (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group et al., 2009). Their mortality rate is two to three times higher, and their life expectancy is markedly lower than their healthy counterparts (Bruno et al., 2009; Patterson et al., 2007; SEARCH for Diabetes in Youth Study Group et al., 2006). The Diabetes Control and Complications Trial (DCCT), a landmark multicenter trial, has shown that tight glycemic control slows or prevents the development of micro- and macro-vascular complications in T1DM (Chrisholm, 1993; Nathan et al., 2005). Furthermore, tight glycemic control has been linked to enhanced quality of life in adolescents with T1DM (Ingerski, Laffel, Drotar, Repaske, & Hood, 2010).

Despite the knowledge gained from the DCCT, poor glycemic control remains prevalent in children and adolescents with T1DM (Hanberger, Samuelsson, Lindblad, Ludvigsson, & Swedish Childhood Diabetes Registry SWEDIABKIDS, 2008; Petitti et

al., 2009). Studies have shown that glycemic control can vary with age, sex, ethnicity, socio-economic status, disease duration, daily insulin dose, type of insulin regimen, frequency of blood glucose monitoring, frequency of clinic visit, blood glucose level, and body mass index (BMI) (DCCT/EDIC Research Group et al., 2009; Helgeson, Honcharuk, Becker, Escobar, & Siminerio, 2010; Paris et al., 2009; Petitti et al., 2009). Factors such as parental education, parental involvement in diabetes management, family dynamics, and adherence may also affect glycemic control (Forsander, Sundelin, & Persson, 2000; Hood, Peterson, Rohan, & Drotar, 2009; Moreland, Tovar, Zuehlke, Butler, Milaszewski, & Laffel, 2004; Petitti et al., 2009), thus suggesting an increased complexity to manage and achieve tight glycemic control in children and adolescents with T1DM.

Vitamin D status is yet another factor that may be linked to glycemic control in children and adolescents with T1DM. Recent studies have found a marked prevalence of vitamin D deficiency in this population (Bener et al., 2008; Di Cesar, Ploutz-Snyder, Weinstock, & Moses, 2006; Greer et al., 2007; Svoren, Volkening, Wood, & Laffel, 2009). Furthermore, there has been increasing evidence linking vitamin D deficiency to the increased risk of T1DM, impaired glucose metabolism, and altered immune responses *in vitro* and *in vivo* (Bouillon et al., 2008). Both glucose metabolism and immune responses are altered in T1DM (Kaufman, 2008). Therefore, it is both pertinent and timely to examine the relationships among vitamin D status, glycemic control, and inflammatory cytokines in metabolically stable children and adolescents with T1DM.

Vitamin D Deficiency in T1DM

There are few studies in the literature that describe the prevalence of vitamin D deficiency in individuals with TIDM (Bouillon et al., 2008; Greer et al., 2007; Svoren et al., 2009). In the U.S., Svoren and colleagues evaluated vitamin D status in 128 children and adolescents with TIDM (mean age 10.8 ± 4.3 years; mean disease duration 4.1 ± 5.6 years) residing in Boston, Massachusetts. Vitamin D deficiency (25-hydroxyvitamin D less than 50nmol/L) and insufficiency (25-hydroxyvitamin D equal or more than 50nmol/L and less than 75nmol/L) was prevalent in 15.0% and 61.0% of the patients, respectively. Vitamin D deficiency was more prevalent among adolescents 12-18 years of age as compared to the younger age group, and among patients with longer disease duration as compared to those with shorter disease duration (Svoren et al., 2009).

These data are comparable to the prevalence of vitamin D deficiency in healthy children and adolescents residing in the U.S. According to the National Health and Nutrition Examination Survey (NHANES) 2001-2004, vitamin D deficiency (defined in this study as 25-hydroxyvitamin D less than 37nmol/L) was found to affect 9.0% of the pediatric population (aged 1-21 years) representing 7.6 million U.S. children and adolescents. In addition, vitamin D insufficiency (defined in this study as 25-hydroxyvitamin D equal or more than 37nmol/L and less than 72nmol/L) was evident in 61.0% of the pediatric population representing 50.8 million U.S. children and adolescents. Vitamin D deficiency was more frequent in non-Hispanic Blacks as compared to Hispanics and non-Hispanic Whites, and in adolescents aged 13-21 years as compared to the younger age group (Kumar, Muntner, Kaskel, Hailpern, & Melamed, 2009). Furthermore, an observational study including 382 healthy children and

adolescents (aged 6-21 years) residing in Philadelphia, PA, found hypovitaminosis D (25-hydroxyvitamin D less than 75nmol/L) in 55.0% of study subjects (Weng et al., 2007). Hypovitaminosis D was evident in more than 90.0% of Blacks during winter.

Vitamin D Status and Risk of Developing T1DM

Evidence supporting the link between vitamin D deficiency and the increased incidence of T1DM comes from ecological correlations, animal studies, and population-based epidemiological studies. Ecologically, the highest incidence of T1DM was observed in countries with the furthest distance from the equator where low ultraviolet B radiation results in low vitamin D cutaneous synthesis. Alternatively, the incidence of T1DM approaches zero in countries with high ultraviolet B radiation (Mohr et al., 2008). In addition, the incidence of T1DM has been suggested to follow a seasonal pattern, with the highest rate observed during winter at the time of lowest vitamin D cutaneous synthesis, and the lowest rate observed during summer at the time of highest vitamin D cutaneous synthesis (Levy-Marchal et al., 1995).

Animal studies, particularly experiments in non-obese diabetic (NOD) mice, have provided the strongest evidence supporting the link between vitamin D deficiency and the increased incidence of T1DM. High doses of $1,25(OH)_2D$ or its analogs, given to young NOD mice, reduced the incidence of insulitis (a histological lesion in pancreatic islets of Langerhans caused by infiltrating immune cells which precedes the clinical presentation of diabetes) and prevented T1DM (Gysemans et al., 2005; Mathieu et al., 1992; Giulietti et al., 2004; Gregori, Giarratana, Smiroldo, Uskokovic, & Adorini, 2002; Mathieu, Waer, Laureys, Rutgeerts, & Bouillon, 1994). Also, high doses of vitamin D_3 analog and

cyclosporine A given to NOD mice with insulitis, halted the progression of insulitis to an overt clinical disease (Casteels et al., 1998).

Observational studies in humans have linked the intake of vitamin D-containing supplements or vitamin D-rich food during pregnancy to the decreased development of diabetes-related auto-antibodies in offsprings throughout the first year of life (Brekke & Ludvigsson, 2007; Fronczak et al., 2003). Similarly, a large cohort epidemiologic study, using data from the 1966 Northern Finland Birth Cohort, suggested an association between vitamin D supplementation (2000 IU daily) during the first year of life and the decreased incidence of T1DM (86.0% reduction) after 30 years of follow-up (Hypponen, Laara, Reunanen, Jarvelin, & Virtanen, 2001).

Vitamin D Status and Glucose Metabolism

Evidence supporting the link between vitamin D status and glucose metabolism comes from both animal and human studies examining the effect of vitamin D deficiency on insulin secretion and sensitivity. Animal studies have shown that $1,25(OH)_2D$ may be locally produced by pancreatic β-cells. Furthermore, $1,25(OH)_2D$ increases intracellular free Ca^{2+} and subsequently increases insulin secretion in pancreatic β-cells *in vitro* (Norman et al., 1980; Sergeev & Rhoten, 1995; G. G. Schwartz et al., 2004).

In humans, there is increasing evidence to support the link between vitamin D deficiency and altered glucose metabolism in both children and adults. In a group of obese children and adolescents, a 10-unit increase in serum 25-hydroxyvitamin D (nmol/L) was linked to a 0.5% (P=0.015) and 0.4% (P=0.025) decrease in fasting glucose level (mmol/L) in boys and girls, respectively (Delvin et al., 2010). In a similar cohort of obese children and adolescents, 25-hydroxyvitamin D was inversely associated with both

HbA1c (r =−0.23, P=0.01) and fasting glucose (r=–0.20, P<0.001) (Alemzadeh, Kichler, Babar, & Calhoun, 2008; Johnson, Nader, Weaver, Singh, & Kumar, 2010). Furthermore, cross-sectional studies including both non-diabetic elderly and adults with impaired glucose tolerance have shown that 25-hydroxyvitamin D levels are associated with 1-hour glucose concentration (r=-0.23, P<0.01) and total insulin concentration (r=-0.18 to -0.23, P<0.05), and positively associated with insulin sensitivity index (r=0.46, P=0.0007) during a standard 75g oral glucose tolerance test (Baynes, Boucher, Feskens, & Kromhout, 1997; Chiu, Chu, Go, & Saad, 2004).

The following three sections provide an overview on the immune modulatory role of vitamin D *in vitro* and *in vivo,* the link between inflammation and micro- and macro-vascular complications in T1DM, and the link between pro- and anti-inflammatory cytokines and glycemic control in T1DM.

Vitamin D Status and Immune Modulation

Vitamin D is an immune modulatory hormone that exerts its effect on the immune system by interacting with vitamin D receptors found in antigen presenting cells, dendritic cells, activated T cells and B cells, monocytes, and macrophages (van Etten & Mathieu, 2005). $1,25(OH)_2D$ directly inhibits T cells proliferation, inhibits dendritic cells differentiation and maturation, down-regulates the expression of MHC class II, co-stimulatory molecules (CD40, CD80, and CD86) and interleukin (IL)-12, and enhances the production of anti-inflammatory cytokines including IL-10, thereby inhibiting dendritic cell-dependent T cell activation *in vitro* (Bouillon et al., 2008; van Etten & Mathieu, 2005). The consequential down-regulation of IL-12 and up-regulation of IL-10 alter the cytokine milieu and shift the differentiation of CD4+ T cells towards a T helper

type 2 (Th2) phenotype, thus inhibiting the production of Th1 cytokines interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and IL-2 while promoting the production of Th2 IL-4, IL-5, and IL-10 (Boonstra et al., 2001; Bouillon et al., 2008). Furthermore, $1,25(OH)_2D$ indirectly impedes the activation of immune-related transcription factors, nuclear factor of activated T cells, nuclear factor *κappa* B ($NF_{\kappa}B$), and activating protein-1, thereby decreasing the production of IL-8, IL-12, IL-2, and IL-4 *in vitro* (Alroy, Towers, & Freedman, 1995; D'Ambrosio et al., 1998; Harant, Wolff, & Lindley, 1998; Staeva-Vieira & Freedman, 2002; Takeuchi et al., 1998; Towers, Staeva, & Freedman, 1999; Yu, Bellido, & Manolagas, 1995).

The immune modulatory role of vitamin D has been examined, as previously described, in NOD mice where treatment with vitamin D or its analogs halted or slowed the progression of the disease. Similarly, the immune modulatory role of vitamin D was examined *in vitro* on the expression of inflammatory cytokines by freshly isolated monocytes from patients with type 2 diabetes mellitus (T2DM), T1DM and healthy controls. The addition of vitamin D to activated monocytes down-regulated the expression of TNF-α, IL-6, IL-1, and IL-8 in monocytes from both T2DM and T1DM patients. However, only the down-regulation of IL-8 in monocytes from T1DM patients reached statistical significance ($P<0.05$). $1,25(OH)_2D_3$ had no effect on IL-10 expression in either group (Giulietti et al., 2007).

In humans, there have been no studies examining the effect of vitamin D supplements on systemic markers of inflammation in T1DM. However, this effect has been observed in patients with other inflammatory conditions such as end-stage renal disease, in elderly women post hip-fracture, in patients with chronic kidney disease, and

in patients with congestive heart failure (Stubbs, Idiculla, Slusser, Menard, & Quarles, 2010; Miller et al., 2007; Neves et al., 2010; Schleithoff et al., 2006).

Inflammation and T1DM Complications

T1DM is a pro-inflammatory state characterized by increased systemic markers of inflammation and monocyte activity, both of which have been linked to the increased risk of micro- and macro-vascular complications (Devaraj et al., 2006; Devaraj et al., 2007; Schalkwijk et al., 1999). *In vitro* studies have reported increased monocyte activity in T1DM patients as compared to healthy controls. This increased monocyte activity was more prominent in T1DM patients with micro-vascular complications. In monocytes of T1DM patients with micro-vascular complications, there was a significant increase of toll-like receptor 2 (TLR2) and TLR4 surface expression as compared to monocytes from T1DM patients with no micro-vascular complications and healthy controls ($P<0.01$) (Devaraj, Jialal, Yun, & Bremer, 2010). The increase in TLR2 and TLR4 surface expression is significantly and positively associated with increased NFκB activity which contributes to the increased monocytic release of IL-1β, IL-1, IL-6, and TNF-α (Devaraj et al., 2007; Devaraj et al., 2010).

Cross-sectional studies and prospective observational studies of adolescents and adults with T1DM have reported significant associations between progressive nephropathy and elevated systemic levels of pro-inflammatory cytokines including soluble intercellular adhesion molecule-1, C-reactive protein, soluble tumor necrosis factor-alpha receptor-1, and vascular cell adhesion molecule-1 (Clausen et al., 2000; Lin et al., 2008; Saraheimo, Teppo, Forsblom, Fagerudd, & Groop, 2003; Zoppini et al., 2001). A cross-sectional examination of the follow-up data of the EURODIAB

Prospective Complications Study, showed a significant association between markers of inflammation (C-reactive protein, IL-6, and TNF), combined in a Z-score, and albuminuria, retinopathy, and coronary artery disease in adults with T1DM (Schram et al., 2005).

Moreover, case-control studies have reported significantly increased systemic levels of C-reactive protein and IL-6 in adolescents with T1DM as compared to healthy controls ($P<0.0001$) (Mangge et al., 2004; Snell-Bergeon et al., 2010). In one of the case-control studies, elevated levels of C-reactive protein and IL-6 were independent of race/ethnicity, sex, hyperglycemia, and obesity, thus suggesting the presence of alternative mechanisms that may be related to elevated levels of C-reactive protein and IL-6 in this population (Snell-Bergeon et al., 2010). C-reactive protein has been associated with systemic inflammation and is considered a systemic predictor of early atherosclerotic lesions and subsequent risk of coronary artery disease (Hayaishi-Okano et al., 2002; Ridker, Rifai, Rose, Buring, & Cook, 2002).

Inflammation and Glycemic Control

There is accumulating evidence that systemic markers of inflammation (e.g. C-reactive protein, IL-6, IL-8, and IL-18) are related to glycemic control in T1DM (Erbagci, Tarakcioglu, Coskun, Sivasli, & Sibel Namiduru, 2001; Foss-Freitas, Foss, Rassi, Donadi, & Foss, 2008; Lo, Lin, & Wang, 2004; Van Sickle et al., 2009; Zozulinska, Majchrzak, Sobieska, Wiktorowicz, & Wierusz-Wysocka, 1999; Scholin et al., 2004). However, to my knowledge, only one study examined the association between IL-10 and HbA1c in T1DM. Sanda and colleagues found an association between higher mean number of IL-10 producing cells, measured at the time of T1DM diagnosis, and

improved glycemic control (r^2=0.68; P=0.08) measured three months after diagnosis (Sanda, Roep, & von Herrath, 2008).

Aims and Hypotheses

Accordingly, the purpose of this study was to examine the relationship between 25-hydroxyvitamin D and HbA1c; and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. Children and adolescents with less than one year duration of the disease has been excluded, since they may experience partial remission and thus may exhibit a different immunologic profile as compared to patients with longer disease duration (Muhammad, Swift, Raymond, & Botha, 1999). The following aims were identified:

1. Quantify the relationship between 25-hydroxyvitamin D and HbA1c excluding inflammatory cytokines (IL-6, IL-8, and IL-10).

   Hypothesis: 25-hydroxyvitamin D is inversely related to HbA1c.
2. Quantify the relationship between 25-hydroxyvitamin D and inflammatory cytokines (IL-6, IL-8, and IL-10) excluding HbA1c.

   Hypothesis: 25-hydroxyvitamin D is inversely related to IL-6 and IL-8, and directly related to IL-10.
3. Quantify the relationship between inflammatory cytokines (IL-6, IL-8, and IL-10) and HbA1c excluding 25-hydroxyvitamin D.

   Hypothesis: IL-6 and IL-8 are directly related to HbA1c. IL-10 is inversely related to HbA1c.

4. Quantify the total indirect effect and specific indirect effects of 25-hydroxyvitamin D on HbA1c through three mediators (IL-6, IL-8 and IL-10). Hypothesis: IL-6, IL-8 and IL-10 mediate the relationship between 25-hydroxyvitamin D and HbA1c.

In addition, if a direct and non-linear relationship between 25-hydroxyvitamin D and HbA1c exist, then:

5. Determine a threshold value for minimum mean 25-hydroxyvitamin D level beyond which greater improvements in HbA1c may be expected.

Conceptual Framework

The marked prevalence of vitamin D deficiency in children and adolescents with T1DM, and the hypothesized relationships among vitamin D status, glycemic control, and inflammatory cytokines in metabolically stable children and adolescents with T1DM, outline the conceptual framework of this proposal. 25-hydroxyvitamin D, the functional indicator of vitamin D status, was selected as the primary independent variable (Heaney, Dowell, Hale, & Bendich, 2003). HbA1c, the standardized index of glycemic control, was the proposed primary outcome (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group et al., 2009; Goldstein et al., 1994; Steffes et al., 2005). Pro-inflammatory cytokines IL-6 and IL-8, and the anti-inflammatory cytokine IL-10, which reflect the state of inflammation in T1DM, were selected as hypothetical mediators that may be related to both 25-hydroxyvitamin D and HbA1c.

Dependent Variable - HbA1c

In the mid 1970s, the relationship between HbA1c and mean blood glucose level was established. In the mid 1980s HbA1c was widely used as the standardized index of glycemic control. In the 1990s, the DCCT study established HbA1c as a measure of risk for the development of micro- and macro-vascular complications in T1DM (DCCT/EDIC Research Group et al., 2009; Goldstein et al., 1994; Steffes et al., 2005). Therefore, the use of HbA1c in this study provides a clinically meaningful indicator for glucose control and a common ground for comparability with previously reported data in diabetes research.

According to the American Diabetes Association (ADA) guidelines, age-specific HbA1c target values are 7.5% to 8.5% for ages 0-6 years, less than 8.0% for ages 6-12 years, and less than 7.5% for ages 12-18 years (Silverstein et al., 2005). Glycemic control has been classified as "good" in individuals who met their target HbA1c, "intermediate" in individuals with HbA1c between their target HbA1c and 9.5% or "poor" in individuals with HbA1c greater than 9.5% (Petitti et al., 2009). Studies have shown that age, sex, ethnicity, socio-economic status, disease duration, daily insulin dose, type of insulin regimen, frequency of blood glucose monitoring, frequency of clinic visit, blood glucose level, and BMI explain a considerable percent of variance in HbA1c (DCCT/EDIC Research Group et al., 2009; Forsander et al., 2000; Helgeson et al., 2010; Hood et al., 2009; Moreland, Tovar, Zuehlke, Butler, Milaszewski, & Laffel, 2004; Paris et al., 2009; Petitti et al., 2009).

Independent Variable - 25-hydroxyvitamin D

In 1997, serum 25-hydroxyvitamin D level was accepted as the functional indicator of vitamin D status by the Food and Nutrition Board of the Institute of Medicine (IOM) (Heaney et al., 2003). Serum 25-hydroxyvitamin D has a relatively long half-life of 2-3 weeks and is considered the best estimate of vitamin D supply from both cutaneous synthesis and dietary intake (Norman, 2008; Prentice, Goldberg, & Schoenmakers, 2008). It is well established that serum 25-hydroxyvitamin D concentration varies with age, gender, ethnicity, skin pigmentation, BMI, season, and latitude (Compher, Badellino, & Boullata, 2008; Jacques et al., 1997; Macdonald et al., 2008; Yetley, 2008).

To date, there is still no consensus between the IOM and vitamin D experts on the cut off point for vitamin D sufficiency (Looker et al., 2011; Holick et al., 2011). Recently, the IOM defined four categories of vitamin D status: a) at risk of vitamin D deficiency (25-hydroxyvitamin D less than 30nmol/L), b) at risk of vitamin D inadequacy (25-hydroxyvitamin D between 30 and 49nmol/L), c) sufficient in vitamin D (25-hydroxyvitamin D between 50 and 125nmol/L), and d) possibly harmful vitamin D (25-hydroxyvitamin D greater than 125nmol/L) (IOM, 2010). On the other hand, the Endocrine Society in consensus with vitamin D experts classify vitamin D status into 3 categories: a) deficiency (25-hydroxyvitamin less than 50nmol/L), b) insufficiency (25-hydroxyvitamin D equal or more than 50nmol/L and less than 75nmol/L) and c) sufficiency (25-hydroxyvitamin D equal or more than 75nmol/L and less than 250nmol/L) (Holick, 2007; Holick, 2008; Prentice et al., 2008; Rovner & O'Brien, 2008; Holick et al., 2011).

Pro-Inflammatory Cytokine IL-6

IL-6 is a pro-inflammatory cytokine that has been associated with the pathogenesis of T1DM, hyperglycemia, and glycemic control (Choudhary & Ahlawat, 2008; Rosa et al., 2008; Targher, Zenari, Bertolini, Muggeo, & Zoppini, 2001; Mohamed-Ali, Armstrong, Clarke, Bolton, & Pinkney, 2001; Targher et al., 2001). Furthermore, studies have reported associations between elevated systemic levels of IL-6 and the increased risk of micro- and macro-vascular complications in T1DM (Devaraj et al., 2007; Schram et al., 2005). Not only this, systemic levels of IL-6 have been associated with disease severity in patients with T2DM, septic shock, trauma, severe acute pancreatitis, and cardiogenic shock (Berney et al., 1999; Choudhary & Ahlawat, 2008; Gebhard et al., 2000; Geppert et al., 2002; Martin, Boisson, Haccoun, Thomachot, & Mege, 1997). In addition, IL-6 has a relatively longer half-life than other pro-inflammatory cytokines including TNF-α and IL-1β (Hirasawa, Oda, & Nakamura, 2009; Oda et al., 2005). As such, IL-6 has been selected as a hypothetical mediator that may be related to both vitamin D status and glycemic control in metabolically stable children and adolescents with T1DM.

Pro-inflammatory Cytokine IL-8

Studies have found a consistent link between elevated systemic levels of IL-8 and hyperglycemia, poor glycemic control, and diabetes complications in patients with T1DM (Erbagci et al., 2001; Foss-Freitas et al., 2008; Lo et al., 2004; Van Sickle et al., 2009; Zozulinska et al., 1999). IL-8 is produced in response to other pro-inflammatory cytokines, such as IL-1β and TNF-α, by a series of cells including monocytes, macrophages, T lymphocytes, neutrophils, fibroblasts, keratinocytes, hepatocytes,

chondrocytes, endothelial cells, glioblastoma cells, and mesothelial cells (Lo et al., 2004). An acute increase in pro-inflammatory cytokines induces intracellular IL-8 mRNA which reaches maximal expression within four hours of cell stimulation and gradually decreases thereafter. Sustained exposure to IL-1β has been shown to stabilize intracellular IL-8 mRNA expression which may explain the previously reported increase in serum concentration of IL-8 in patients with T1DM. As such, IL-8 has been selected as a hypothetical mediator that may be related to both vitamin D status and glycemic control in metabolically stable children and adolescents with T1DM.

Anti-Inflammatory Cytokine IL-10

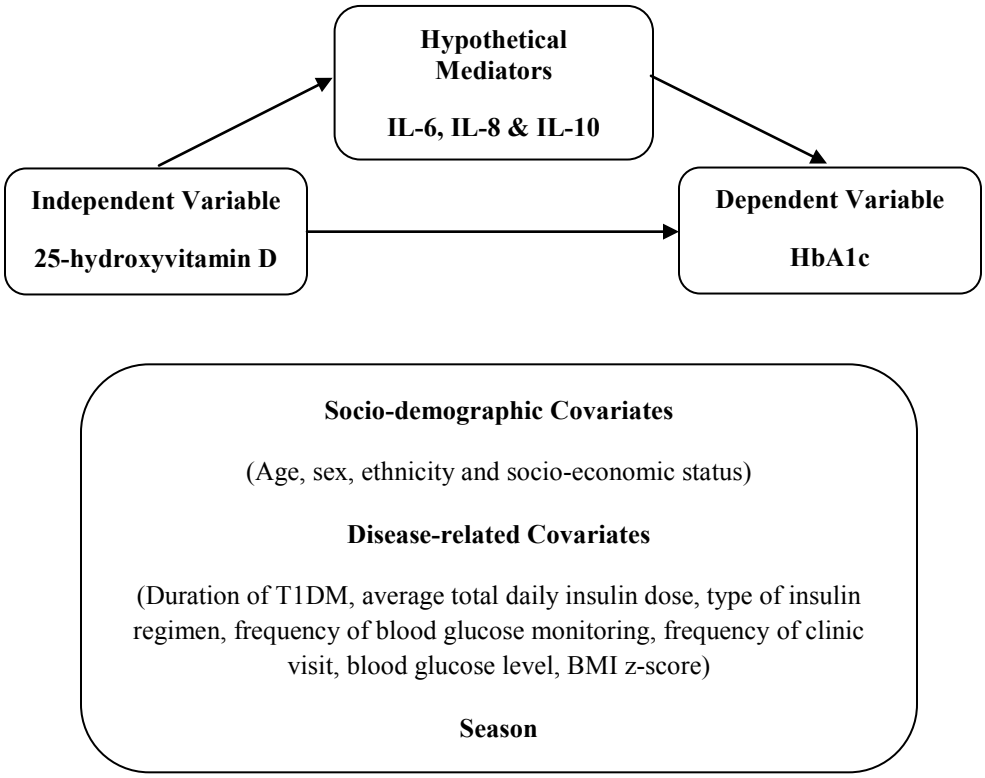
IL-10 is an anti-inflammatory cytokine that is produced by a variety of cells including monocytes and T cells. It is known to inhibit Th1 responses, inhibit pro-inflammatory cytokines, and increase the expression of Foxp3+ in T cells, thus changing their phenotype into T regulatory cells. Furthermore, studies have shown that vitamin D enhances the development of IL-10 producing cells which then reduces the number of IL-6 producing cells (Correale, Ysrraelit, & Gaitan, 2009). To date, there is only one study that has reported an association between the frequency of IL-10 producing cells at the time of T1DM diagnosis and glycemic control measured three months thereafter (Sanda et al., 2008). Given its well established association with vitamin D, IL-10 has been selected as a hypothetical mediator that may be related to both vitamin D status and glycemic control in metabolically stable children and adolescents with T1DM.

Significance of the Study

This nursing research is unique; it integrates both clinical and bench research, where biological markers have been used to address important clinical research questions

on the relationships among vitamin D status, glycemic control, and inflammatory cytokines in metabolically stable children and adolescents with T1DM. The knowledge gained from this research may guide the direction of future research and may be useful for developing clinical guidelines to reduce the magnitude of vitamin D deficiency and insufficiency in children and adolescents with T1DM.

Figure 1.1 This figure is a visual representation of the hypothesized relationships among 25-hydroxyvitamin D, HbA1c, and inflammatory cytokines IL-6, IL-8, and IL-10, adjusted for important covariates.



CHAPTER TWO: REVIEW OF LITERATURE

The purpose of this study was to examine the relationship between 25-hydroxyvitamin D and HbA1c; and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. Chapter two starts with overviews on the incidence and prevalence of T1DM in the U.S., pathogenesis of T1DM, clinical presentation and honeymoon phase, medical management of T1DM, and lessons learned from the Diabetes Control and Complications Trial (DCCT). The subsequent section provides overviews on predictors of glycemic control in children and adolescents with T1DM and glycated hemoglobin (HbA1c). Section three reviews sources of vitamin D, predictors of vitamin D status, vitamin D metabolism, the suggested link between vitamin D deficiency and the increased risk of developing T1DM, the role of vitamin D in glucose homeostasis, and the role of vitamin D in immune modulation.

Section One

Incidence and Prevalence of T1DM in the US

T1DM is one of the leading chronic diseases of childhood and adolescence, affecting one in every 400 to 600 children and adolescents in the United States (SEARCH for Diabetes in Youth Study Group, 2006; Centers for Disease Control and Prevention [CDC], 2007). In the 1990's, the World Health Organization (WHO) Multinational Project for Childhood Diabetes (WHO DIAMOND Project) reported a mean annual increase in T1DM incidence of 5.5% among children and adolescents, 0-14 years, residing in the U.S. (DIAMOND Project Group, 2006). It was not until the year 2000 that the SEARCH for Diabetes in Youth Study (SEARCH) (a population-based, observational

study of physician-diagnosed diabetes among youth <20 years of age) was initiated to estimate the overall prevalence and incidence of diabetes in children and adolescents residing in the U.S. SEARCH, which is still ongoing till present, collected data from six centers located in California, Colorado, Hawaii, Ohio, South Carolina, and Washington. In 2001, SEARCH estimated the overall prevalence of diabetes in children and adolescents at 1.82 cases per 1000 youth; of which 0.76 cases per 1000 and 2.80 cases per 1000 were children 0-9 years and adolescents 10-19 years with T1DM, respectively (The SEARCH for Diabetes in Youth Study Group, 2006). The prevalence of T1DM was 2.0 cases per 1000 in non-Hispanic Whites 0-19 years, 0.57 cases per 1000 in African Americans 0-9 years, and 2.04 cases per 1000 in African Americans 10-19 years (Bell et al., 2009; Mayer-Davis et al., 2009). Furthermore, diabetes was found in 781 out of 641,414 Hispanic Americans, of which 99.3% of children younger than 10 years, 85.5% of adolescents 10-14 years, and 71.3% of adolescents 15-19 years had T1DM (Lawrence et al., 2009). Furthermore, in 2002 and 2003, the overall incidence rate of diabetes was 24.3 cases per 100 000 person-years; with the highest incidence rate observed among adolescents 10-14 years (33.9 cases per 100 000 person-years) and non-Hispanic Whites (26.1 cases per 100 000 person-years) (Writing Group for the SEARCH for Diabetes in Youth Study Group et al., 2007).

In addition to SEARCH, several other population-based diabetes registries have been established in the U.S. including the Wisconsin Diabetes Registry, Chicago Childhood Diabetes Registry, Allegheny County Childhood-Onset T1DM Registry, and the Philadelphia Pediatric Diabetes Registry (Barcelo, Bosnyak, & Orchard, 2007; Lipman et al., 2006; Palta & LeCaire, 2009; Smith, Drum, & Lipton, 2007; Lipman et al.,

2009). Of relevance to this study, the Philadelphia Pediatric Diabetes Registry demonstrated that the age-adjusted incidence rate of T1DM in children 0-14 years was 18.2 per 100,000/year in White children, 14.0 per 100,000/year in Black children and 14.0 per 100,000/year in Hispanic children from 2000 to 2004 (Lipman et al., 2009). Interestingly, the age-adjusted incidence rate of T1DM in White children, 0-14 years, residing in Philadelphia has increased from 12.8 per 100,000/year from 1995 to 1999 to 18.2 per 100,000/year from 2000 to 2004 (Lipman et al., 2006; Lipman et al., 2009).

Pathogenesis of T1DM

T1DM is characterized by the immune destruction of pancreatic β-cells, slow and progressive loss of endogenous insulin production and subsequent hyperglycemia (Zipris, 2009; Kaufman, 2008). An inflammatory model has been suggested for the pathogenesis of T1DM (Bergholdt et al., 2004). Environmental factors, most likely common viruses, induce a T cell mediated destruction of the β-cells of the pancreas in genetically susceptible individuals. CD8+ T cells recognize MHC I restricted presentation of β-cell antigen and cause limited β-cell damage via the release of cytotoxic cytokines. Limited β-cell damage initiates the release of glycosylated immature β-cell components, such as insulin or glutamic acid decarboxylase (GAD) not previously "seen" by the immune system and therefore recognized by the immune system as non-self antigens. Glycosylated immature β-cells components are taken up by dendritic cells in the islets and transported to regional pancreatic lymph nodes, where antigens are processed and presented to CD4+ T cells. Activated CD4+ T cells will then orchestrate the buildup of specific and non-specific inflammatory cells in the pancreatic islets causing inflammatory insulitis. Pro-inflammatory cytokines induce proapoptotic signaling in β-cells and/or

induce β-cells expression of Fas ligand thus labeling β-cells for MHC II non-restricted CD4+ T cells mediated killing (Bergholdt et al., 2004).

Clinical Presentation and Honeymoon Phase

In T1DM, hyperglycemia becomes evident when 60.0% to 80.0% of pancreatic β-cells are destroyed (Notkins & Lernmark, 2001; Kaufman, 2008). At the time of diagnosis, individuals with T1DM present with an array of clinical symptoms secondary to uncontrolled hyperglycemia including polyuria, polydipsia, polyphagia, blurred vision, fatigue, weight loss, and often ketoacidosis (Kaufman, 2008). Once the T1DM diagnosis is confirmed, exogenous insulin therapy is initiated to prevent rapid and severe dehydration, catabolism, ketoacidosis, and death (Jacobsen, Henriksen, Hother-Nielsen, Vach, & Beck-Nielsen, 2009). Individuals with a clinical diagnosis of T1DM receive exogenous insulin therapy for life.

Interestingly, newly diagnosed children and adolescents with T1DM experience a partial remission phase, known as the honeymoon phase, during the first year after diagnosis. It has been documented that the honeymoon phase affects approximately 50.0% of children diagnosed with T1DM (Muhammad et al., 1999). Patients experiencing partial remission have improved production of endogenous insulin, achieve close to normal blood glucose control, and thus require lower daily insulin dose of less than 0.5unit/kg/day --expected daily insulin dose for children and adolescents with T1DM is approximately 1unit/kg/day (Sanda et al., 2008; Kaufman, 2008). It is therefore suggested that patients experiencing partial remission may not exhibit the same profile of immunologic changes observed in patients who have recovered from remission, and are

beyond the first year of diagnosis. For this reason, children and adolescents with less than one year duration of T1DM have been excluded from this study.

Medical Management of T1DM

Medical management of T1DM is complex, ongoing, and requires the collaboration among the patient, parents, and members of the diabetes healthcare team. The goal of T1DM medical management is to achieve and maintain tight glycemic control while decreasing the frequency of hypoglycemia. The DCCT (1983-1989) firmly established the significance of tight glycemic control in slowing down the development and progression of diabetes related micro- and macro-vascular complications in T1DM (DCCT/EDIC Research Group et al., 2009). Based on these findings, intensive insulin therapy was adopted as the gold standard therapy for achieving tight glycemic control in children and adolescents with T1DM. Intensive insulin therapy may be either multiple daily injections (MDI) of three or more insulin injections per day or continuous subcutaneous insulin infusion (CSII or insulin pump) (Silverstein et al., 2005). To date, randomized controlled trials and observational studies have shown that CSII may be better than MDI in achieving better glycemic control, however, the associated risk of severe and non-severe hypoglycemia remains unchanged (Misso, Egberts, Page, O'Connor, & Shaw, 2010; Sherr, Cengiz, & Tamborlane, 2009). The DCCT reported a three-fold increase in the frequency of severe hypoglycemia associated with intensive insulin therapy (DCCT/EDIC Research Group et al., 2009). Severe hypoglycemia may increase the risk of hypoglycemia unawareness, neurocognitive damage, and emotional morbidity in young children with T1DM (Shalitin & Phillip, 2008).

To balance the risks and benefits of intensive insulin therapy in children and adolescents with T1DM, the ADA designed age-specific glycemic goals. Age-specific glycemic goals for ages 0-6 years, 6-12 years, and 12-18 years are 7.5% to 8.5%, less than 8.0%, and less than 7.5%, respectively (Silverstein et al., 2005). Glycemic control has been classified as "good" in individuals who met their target HbA1c, "intermediate" in individuals with HbA1c between their target HbA1c and 9.5%, or "poor" in individuals with HbA1c greater than 9.5% (Petitti et al., 2009).

From the patient and family's end, self-monitoring of blood glucose (SMBG) serves as a guide for diabetes self-management. As per the ADA guidelines, SMBG should be performed three or more times daily for patients using MDI or CSII (American Diabetes Association, 2010). SMBG provides information on the hour-to-hour effectiveness of insulin therapy, whether glycemic targets are achieved, whether insulin doses need to be adjusted, whether there is an impending risk of hypoglycemia, or whether nutrition or physical therapy needs to be modified (American Diabetes Association, 2010).

Lessons Learned from Diabetes Control and Complications Trial (DCCT)

The DCCT (1983-1989) and subsequently the Epidemiology of Diabetes Interventions and Complications (EDIC; 1994-present) study provided the basis for our understanding of the relationship between glycemic control and the risk of developing diabetes-related micro- and macro-vascular complications later in life. The DCCT study enrolled 1,441 patients with TIDM 13-39 years with mild to moderate retinopathy (disease duration ranged from 1-15 years) that were randomly assigned to either intensive insulin therapy or conventional therapy (one or two insulin injections per day), and then

followed up participants for 6.5 years (Jacobsen et al., 2009; Springer et al., 2006). The intensive treatment group sustained a significantly lower mean HbA1c as compared to the conventional treatment group (7.4% vs. 9.1%, P<0.0001; respectively) and had markedly lower risk for progression of retinopathy and development of micro-albuminuria (urinary albumin excretion ≥40 mg/24 h), clinical proteinuria (urinary albumin excretion ≥300 mg/24 h) and clinical neuropathy (Tamborlane & Ahern, 1997). In 1994, EDIC followed up 96.0% of the surviving cohort from the DCCT. All participants of the conventional treatment group were offered intensive therapy based on the beneficial outcomes of the DCCT. From 1994 till 2005, mean HbA1c was 8.0% in the former conventional treatment group and 7.8% to 8.1% in the former intensive treatment group. Despite equalization of glycemic indices in both treatment groups, the former intensive treatment group sustained a lower cumulative incidence of proliferative retinopathy (21.0%), nephropathy (9.0%), and cardiovascular disease (9.0%) after 30 years of diabetes, as compared to the conventional treatment group (50.0%, 25.0%, and 14.0%, respectively) (DCCT/EDIC Research Group et al., 2009).

Section Two

Predictors of Glycemic Control in Children and Adolescents with T1DM

Despite the lessons learned from DCCT and EDIC, there remain a significant number of children and adolescents with intermediate or even poor glycemic control. A cross-sectional study including 2,837 children and adolescents with T1DM from 22 centers world-wide including North America, Europe, and Japan found good or intermediate glycemic control (HbA1c <8.0%) in 41.0% of children younger than 11 years and only in 29.0% of adolescents 12-18 years (Mortensen et al., 1998). According

to SEARCH study, poor glycemic control (HbA1c >9.5%) was evident in 36.0% of African-Americans, 52.0% of American Indians, 27.0% of Hispanics, 26.0% of Asian/Pacific Islanders, and 12.0% of Whites with T1DM (Petitti et al., 2009). The same study found intermediate or poor glycemic control (HbA1c >7.5 %) in more than 70% of adolescents regardless of their insulin regimen (Paris et al., 2009). These data are concerning and further highlight the complexity of achieving and maintaining tight glycemic control in children and adolescents with T1DM.

Glycemic control has been linked to a series of disease-related and socio-demographic variables (DCCT/EDIC Research Group et al., 2009; Forsander et al., 2000; Helgeson et al., 2010; Hood et al., 2009; Moreland, Tovar, Zuehlke, Butler, Milaszewski, & Laffel, 2004; Paris et al., 2009; Petitti et al., 2009). In a cross-sectional analysis including 300 children and adolescents with T1DM 7-16 years residing in Boston, HbA1c was directly associated with age ($P<0.001$), duration of diabetes ($P< 0.001$), insulin dose ($P<0.001$), and BMI (kg/m^2) ($P<0.001$), and inversely associated with frequency of blood glucose monitoring (FBGM) ($P=0.004$), family structure ($P=0.002$), and father's highest educational level ($P= 0.002$) (Levine et al., 2001). On the other hand, a cross-sectional study examining the effect of ethnicity on HbA1c in 183 children and adolescents with T1DM 1-21 years (99 non-Hispanic Whites and 84 Hispanics), found poorer glycemic control in Hispanics as compared to non-Hispanic Whites (0.45% difference in HbA1c levels, $P=0.02$). In a multivariate model, poor glycemic control was associated with low house-hold income ($P=0.0024$) and was not related to insurance status, parental level of education, family structure, or ethnicity (Gallegos-Macias, Macias, Kaufman, Skipper, & Kalishman, 2003). These data may suggest a significant

impact of family structure, parental level of education, and house-hold income on health-related behaviors including adherence and diabetes self-management, which are very likely to affect glycemic control.

The SEARCH for diabetes in youth, found significant direct associations between HbA1c and age at the time of examination ($P<0.0001$), sex ($P=0.027$), ethnicity ($P<0.0001$), diabetes duration ($P<0.0001$), and BMI percentile ($P=0.0018$), and significant inverse associations between HbA1c and family structure ($P=0.0003$), and parental education ($P<0.0001$) (Paris et al., 2009; Petitti et al., 2009). Of note, HbA1c was lowest in youth receiving CSII than in those receiving MDI; however, both increasing age and infrequent SBGM (≤ 2 times per day) were associated with higher mean HbA1c, regardless of the insulin regimen (Paris et al., 2009). A recent review of the literature by Sherr et al., (2009) showed consistent results from 17 non-randomized pediatric studies regarding the significant reduction in HbA1c in children and adolescents with T1DM receiving CSII versus MDI (Sherr et al., 2009). Moreover, a meta-analysis of 23 randomized controlled trials comparing CSII with MDI in 976 patients with T1DM reported a significantly better HbA1c in the CSII group (weighted mean difference -0.3%; 95% CI -0.1 to -0.4) (Misso et al., 2010). Furthermore, there is increasing evidence to support the association between HbA1c and FBGM and the idea that this relationship may vary considerably with age. A cross-sectional study including 229 children and adolescents 9-15 years attending the Florida Camp for children and youth with diabetes reported significant associations between age and both HbA1c ($r=0.18$, $P=0.02$) and FBGM ($r=-0.16$, $P=0.01$) (Haller, Stalvey, & Silverstein, 2004). Also, a retrospective study of 26,723 children and adolescents 0-18 years with T1DM residing in Germany

found a significant difference in FBGM per age group, with it being highest in children younger than 6 years as compared to children 6-12 years or adolescents older than 12 years (6.0/d vs. 5.3/d vs. 4.4/d, $P<0.001$) (Ziegler et al., 2010). The same study showed a higher FBGM in children on CSII compared to children on MDI or conventional therapy ($P<0.001$). In a multivariate model, after adjusting for covariates of glycemic control, a one-unit increase in FBGM was associated with a 0.20% decrease in HbA1c ($P<0.001$) (Ziegler et al., 2010).

Interestingly, a retrospective chart review, by Hochhauser and his colleagues examined the relationship between age at the time of T1DM diagnosis, gender, and glycemic control in 201 children and adolescents with T1DM (48.0% females). This study found no effect of age at the time of diagnosis on HbA1c; however, girls diagnosed at ages 6-12 years had significantly higher HbA1c levels compared to those diagnosed when they were younger or older and boys of the same age group. Although these findings have not been reported in previous studies, they suggest a significant interactive effect of age at diagnosis and gender on glycemic control in females 6-12 years. This interaction between age at diagnosis and gender may be related to pubertal changes and associated insulin resistance occurring during Tanner stages two to four (Hochhauser, Rapaport, Shemesh, Schmeidler, & Chemtob, 2008).

Other factors that may affect glycemic control include diet and exercise. The relationship between diet and glycemic control in children and adolescents with T1DM is complex simply because diet is individualized and is adjusted according to a patient's food preferences, cultural influences, physical activity patterns, and family eating patterns and schedules (Silverstein et al., 2005). A retrospective study examined the

relationship between HbA1c and diet composition in 532 patients with T1DM who participated in the DCCT, were on intensive insulin treatment, and had complete dietary data over 5 years of follow-up (Delahanty et al., 2009). A diet high in fat and low in carbohydrate was associated with higher HbA1c (P=0.001); however this relationship seemed to be confounded by exercise level, serum triglycerides, and BMI (P=0.02) and was no longer significant after adjustment for baseline HbA1c and concurrent insulin dose. On the other hand, studies examining the relationship between exercise and glycemic control have found weak or no associations. A cross-sectional observational study, part of the Hvidoere Study Group on Childhood Diabetes, including 2,093 adolescents 11-18 years, found no association between physical activity and HbA1c; however more time spent doing school homework ($r=-0.09$; $P<0.001$) and less time spent on the computer ($r=0.06$; $P<0.05$) were associated with lower HbA1c (Aman et al., 2009). A smaller-scale cross-sectional study in 142 children and adolescents with T1DM 6-18 years, found a significant correlation between physical activity and daily insulin dose ($r=-0.193$, $P=0.0014$) but not HbA1c. The same study reported a significant association between BMI and HbA1c ($r=0.277$, $P=0.007$) (Raile et al., 1999). In essence, studies examining the relationship between glycemic control and diet or exercise found either no or weak associations.

Family factors; including family structure, level of parental education, and household income may affect glycemic control in children and adolescents with T1DM (Levine et al., 2001; Paris et al., 2009; Petitti et al., 2009). In line with the previously cited studies, a survey analysis, including 153 youth 8-16 years with T1DM in Boston, found lower HbA1c levels in youth who have less negative affect related to blood glucose

monitoring, whose parents have higher diabetes-specific knowledge, and whose parents have less parental-perceived burden of diabetes management (r^2=0.31; P<0.0001) (Butler et al., 2008). Also, Moreland and colleagues found significant associations between HbA1c and level of child-reported diabetes-specific family conflict, FBGM, and CSII (r^2=0.20, P<0.001) in 153 children and adolescents with T1DM (Moreland, Tovar, Zuehlke, Butler, Milaszewski, & Laffel, 2004).

Insurance status has been consistently and significantly linked to glycemic control in bivariate analyses in children and adolescents with T1DM. However, this relationship loses statistical significance when adjusted for family structure, level of parental education, and household income in multivariate analyses (Gallegos-Macias et al., 2003; Levine et al., 2001; Paris et al., 2009; Petitti et al., 2009). Insurance have been used as a proxy for socio-economic status because of the absence of alternative direct measures of socio-economic status in this study. Furthermore, insurance status may be considered as a proxy for the aforementioned family factors that may affect glycemic control in this population. An interesting study by the Consortium for Quality Improvement in Safety Net Hospitals including 47,978 adults with diabetes (57±13 years) found significant associations between glycemic control and age (P<0.01), race (P<0.01), gender (P=0.01) and health insurance coverage (P=0.01). Poor glycemic control (HbA1c >9.5%) was more common in younger patients (P<0.0001), in patients who were never insured (P=0.004), in non-Hispanic Whites patients (P<0.0001), and in females (P=0.017) (Chew, Schillinger, Maynard, Lessler, & Consortium for Quality Improvement in Safety Net Hospitals, 2008). Future studies will be needed to confirm these results in children and adolescents with T1DM.

Glycated Hemoglobin

Glycated hemoglobin (Hb) is formed by the non-enzymatic binding of glucose to hemoglobin (Peterson et al., 1998). Similarly, HbA1c is formed by the non-enzymatic binding of glucose to hemoglobin A, which comprises approximately 90.0% of the total hemoglobin (Bunn, Haney, Kamin, Gabbay, & Gallop, 1976). Red blood cells are freely permeable to glucose, and therefore the rate of formation of HbA1c is directly proportional to mean blood glucose level (Koenig & Cerami, 1979). Furthermore, red blood cells have an average life span of 120 days; thus HbA1c reflects mean blood glucose level over the preceding two to three months (approximately 120 days) (Saudek, Derr, & Kalyani, 2006). Interestingly, there have been several studies investigating the link between mean glucose level and HbA1c. In adults with diabetes, a one-percent increase in HbA1c was equivalent to a 32 to 36mg/dl increase in mean glucose level (Nathan, Turgeon, & Regan, 2007; Rohlfing, Wiedmeyer, Little, England, Tennill, & Goldstein, 2002b). In studies including children and adolescents with T1DM, a one-percent increase in HbA1c corresponded to an 18 to 19 mg/dl increase in mean glucose level, which implies that a slight improvement in mean glucose level may translate into greater improvements in HbA1c in this young population (Diabetes Research in Children Network (DirecNet) Study Group, Wilson, & Kollman, 2008; Tamborlane et al., 2005).

Historically, HbA1c has been used to determine the extent of glycemic control in persons with a clinical diagnosis of diabetes (Selvin et al., 2010). In the last two decades, it has been used as a measure of risk for future development of micro- and macro-vascular complications in T1DM (DCCT/EDIC Research Group et al., 2009; Goldstein et al., 1994; Steffes et al., 2005). Recently, the ADA has supported the use of HbA1c in

establishing a diabetes diagnosis (American Diabetes Association, 2009). Compared to fasting glucose, HbA1c has higher repeatability (reliability), can be assessed in non-fasting state, and provides a clinically meaningful estimate of both glycemic control and future risk of micro- and macro-vascular complications (Selvin, Crainiceanu, Brancati, & Coresh, 2007; Selvin et al., 2010). Currently, HbA1c point-of-care instruments are available and have been widely used to efficiently evaluate glycemic control in diabetes clinics. According to a recent review by Lenters-Westra and Slingerland, only two point-of-care instruments, Afinion and the DCA Vantage, meet the criteria of the National Glycohemoglobin Standardization Program (NGSP) for clinical accuracy (CV<3%) compared to the conventional laboratory-based ion-exchange high-performance liquid chromatography method used to quantify HbA1c in DCCT (Lenters-Westra & Slingerland, 2010; Rohlfing, Wiedmeyer, Little, England, Tennill, & Goldstein, 2002). The DCA Vantage was used to measure HbA1c in this study.

Section Three

Sources of Vitamin D

Vitamin D (vitamin D without a subscript represents either vitamin D_2 or D_3) is a 9,10-seco steroid that exists in two distinct forms; vitamin D_2 and vitamin D_3. Vitamin D_2 (ergocalciferol) is a 28-carbon molecule derived from the plant sterol ergosterol, whereas vitamin D_3 (cholecalciferol) is a 27-carbon derivative of cholesterol (Hollis, 2008). Humans acquire vitamin D from three different sources including exposure to sunlight, diet, and dietary supplements. Exposure to sunlight contributes to approximately 80.0% to 90.0% of the body's requirement for vitamin D (Holick, 1994). During exposure to sunlight, the ultraviolet B photons (wavelength, 290-315nm) get absorbed by 7-

dehydrocholesterol (provitamin D), a four-member ring steroid, in the epidermis and dermis (Holick, 1994; Webb & Holick, 1988). This causes the provitamin D ring to open at C9-C 10 to yield a 6,7-cis-hexatriene derivative known as previtamin D. Previtamin D is biologically inert and undergoes a temperature-dependent isomerization with shifting of the double bonds followed by a rotation about the single C6-C7 bond to yield cholecalciferol (vitamin D_3), a thermodynamically stable 5,6-cis isomer (Holick, 1994; Webb & Holick, 1988). Cholecalciferol exits the skin into the dermal capillaries where it binds to the vitamin D-binding protein (Holick, 1994; Holick, 2007).

Upon prolonged exposure to sunlight, the skin will convert approximately 15.0% of its total 7-dehydrocholesterol to previtamin D (Holick, 1994). This is particularly essential to prevent vitamin D toxicity. Once previtamin D is formed, it can absorb energy between 240-320nm and can undergo a series of reversible reactions that differ from the cholecalciferol pathway (Holick, 1994; Webb & Holick, 1988). Previtamin D can either reverse back to its parent provitamin D, photoisomerize into the biologically inactive photoisomers lumisterol and tachysterol, or isomerize to form a 6,7-trans isomer tachysterol (Holick, 1994; Webb & Holick, 1988).

Few foods naturally contain vitamin D_3 and the amounts of naturally occurring vitamin D_3 vary widely within human food sources. Dietary sources of vitamin D_3 include oily fish, egg yolks, and fortified products such as margarine, fluid milk, and cereal (Prentice et al., 2008). Vitamin D_2 is found in mushrooms. Fluid milk is the only dairy food that is vitamin D_3 fortified and its content varies significantly from one manufacturer to another (Holick, 2007). It has been reported that meats from poultry, pork, and beef contain small amounts of vitamin D that probably come from vitamin D-

fortified animal feed. Vitamin D_2 is the common form of vitamin D used in over-the-counter vitamin D supplements in the United States, although vitamin D_3 supplements are available (Holick, 2007). Vitamin D from both diet and dietary supplements is absorbed in the small intestine via both the lymphatic system (as part of chylomicrons) and the portal vein (bound to vitamin D-binding protein) to the liver (Brannon, Yetley, Bailey, & Picciano, 2008; Prentice et al., 2008).

Predictors of Vitamin D Status

Melanin is one of the main endogenous factors that regulate the cutaneous synthesis of vitamin D. It is a natural sunscreen that absorbs ultraviolet B radiation between 290 and 320nm (Holick, 1995). The concentration of melanin in the skin is inversely proportional to the cutaneous production of vitamin D. Sunscreen with a sun protection factor (SPF) 15 is an exogenous factor that absorbs 99.0% of ultraviolet B photons, and therefore decreases the cutaneous synthesis of vitamin D by approximately 99.0%. Individuals with skin types five (dark intermediate) and six (dark) have a natural SPF of approximately eight to 30 because of the high melanin concentration in their skin, and therefore are considered at a high risk for developing vitamin D deficiency (Holick, 1994).

Age is another endogenous factor that regulates the cutaneous synthesis of vitamin D. Studies have shown that after 20 years of age, skin thickness as well as the concentration of 7-dehydrocholesterol decreases linearly with age (Holick, 1995). The amount of 7-dehydrocholesterol in the epidermis of a 70 years old is approximately 25.0% of that in a 20 years old (Holick, 2008).

Season is an exogenous determinant of the cutaneous synthesis of vitamin D. Studies have reported an increased prevalence of rickets during the winter season and its decline during summer and fall (Holick, 1995). During the winter, people are more likely to stay indoors and wear clothing that covers the entire surface area of their skin, thereby decreasing their exposure to sunlight. Furthermore, during fall and winter seasons, the zenith angle of sunlight is so oblique that it increases the absorption of ultraviolet B radiation in the earth's ozone layer and decreases the amount of ultraviolet B radiation reaching the surface of the earth. Thus, there is little or no production of vitamin D_3 in the skin (Holick, 1995; Holick, 1996). Furthermore, the efficiency of cutaneous conversion of 7-dehydrocholesterol to previtamin D has been associated with latitude, month, and time of the day. At 42°N latitude, maximum cutaneous conversion of 7-dehydrocholesterol to previtamin D was reached during June and July followed by a decline after August reaching less than 4.0% during October (Holick, 1995). At the same latitude, there was no detectable production of previtamin D from November through March. In areas closer to the equator, such as Los Angeles (34°N) and Puerto Rico (18°N) previtamin D production has been shown to occur throughout the year (Holick, 1995).

Vitamin D Metabolism

Vitamin D, whether synthesized in the skin or ingested in the diet, is biologically inert. It is transported to the liver mostly bound to vitamin D-binding protein which circulates at a much higher concentration than vitamin D and its metabolites (Prentice et al., 2008). In the liver, vitamin D undergoes first hydroxylation to 25-hydroxyvitamin D which is biologically inert and is the major circulating form of vitamin D with a relatively long half-life of two to three weeks. 25-hydroxyvitamin D undergoes a second

hydroxylation in the kidney by the enzyme 1α-hydroxylase to form 1, 25$(OH)_2$D which has a half-life in the circulation of approximately four to six hours (Prentice et al., 2008). 1, 25$(OH)_2$D binds with high affinity to specific nuclear receptors; the vitamin D receptors, and induces genomic responses by regulating as many as 500 genes in the human genome (Holick, 1994; Norman, 2008; Prentice et al., 2008; Norman, Frankel, Heldt, & Grodsky, 1980). 1,25$(OH)_2$D also induces nongenomic responses by rapidly activating a variety of signal transduction pathways at or near the plasma membrane. Vitamin D receptors have been detected in thirty-six tissues, including intestine, skin, kidneys, bone, parathyroid glands, pancreas, ovary, uterus, placenta, breast, lymphocytes, monocytes, macrophages, and embryonic liver and muscle (Holick, 1994; Norman, 2008; Prentice et al., 2008). They have also been found in tumor cell lines including breast cancer, malignant melanoma, and leukemia cells (Holick, 1994). Under controlled experimental conditions, the interaction of 1,25$(OH)_2$D with vitamin D receptors in tissues can result in a large array of different effects such as stimulating calcium-binding-protein activity in certain regions of the brain, inhibiting interleukin IL-2 production in activated T lymphocytes, enhancing IL-1 production in activated monocytes, inhibiting parathyroid hormone synthesis, enhancing thyrotropin action, and inhibiting the proliferative activity of cultured human fibroblasts (van Etten & Mathieu, 2005).

1α-hydroxylase is also present in extrarenal tissues, including osteoclasts, skin, macrophages, placenta, colon, brain, prostate, endothelium, and parathyroid glands; however, its expression varies with the physiologic state of the individual (Brannon et al., 2008). The extrarenal production of 1,25$(OH)_2$D generates its effect locally and is

thought to play an important role in cell differentiation, proliferation, and immune function (Norman, 2008).

25-hydroxyvitamin D can undergo a different type of hydroxylation in the renal tissues to form 24,25$(OH)_2$D (Prentice et al., 2008). 24,25$(OH)_2$D is the first product of a metabolic pathway needed to inactivate and degrade 25-hydroxyvitamin D (Prentice et al., 2008). It is found in the circulation bound to vitamin D-binding protein and is thought to affect osteoblast and osteoclast function, bone strength, and parathyroid gland function.

Vitamin D Status and Risk of T1DM

The strongest evidence suggesting a link between vitamin D deficiency and the increased risk of developing T1DM comes from both animal and human studies. In young NOD mice with no evidence of insulitis (a histological lesion in pancreatic islets of Langerhans caused by infiltrating immune cells which precedes the clinical presentation of diabetes) treatment with high doses of 1,25$(OH)_2$D decreases chemokine and cytokine expression by the pancreatic islets and therefore reduces the incidence of insulitis (Gysemans et al., 2005; Mathieu et al., 1992). Treating NOD mice with high doses of 1,25$(OH)_2$D or its analogs at an early age can prevent T1DM, while having a vitamin D deficient state at an early age aggravates it (Giulietti et al., 2004; Gregori et al., 2002; Mathieu et al., 1994). In adult NOD mice, the combined treatment of vitamin D_3 analog and cyclosporine A can prevent the progression of insulitis to an overt clinical disease and shift the cytokine expression in the pancreas from Th1 to Th2 (Casteels et al., 1998). At the cellular level, treating adult NOD mice with vitamin D_3 analog inhibits IL-

12 production, blocks pancreatic infiltration of Th1 cells, enhances CD4+CD25+ regulatory cells, and arrests the progression of T1DM (Gregori et al., 2002).

In humans, controversial reports exist on the association between vitamin D receptor gene polymorphisms including FokI, BsmI, ApaI, and TaqI, and the increased incidence of T1DM (Ban et al., 2001; Chang et al., 2000; Fassbender et al., 2002; McDermott et al., 1997; Nejentsev et al., 2004; Pani et al., 2000; Turpeinen et al., 2003). Alternately, human population studies have shown a link between the intake of vitamin D-containing supplements or vitamin D-rich food during pregnancy with the decreased development of diabetes-related auto-antibodies in offspring throughout the first year of life (Brekke & Ludvigsson, 2007; Fronczak et al., 2003). A large cohort epidemiological study by Hypponen and colleagues suggested a protective role of high-dose vitamin D supplement (2000 IU daily) intake during the first year of life as manifested by a decreased incidence (86.0%) of T1DM over 30 years of follow up (Hypponen et al., 2001). One open-label randomized trial, explored the effect of 0.25µg Calcitriol (vitamin D analog) given every other day to a group of 34 children with new onset T1DM, on residual pancreatic β-cell function after one year of treatment (Pitocco et al., 2006). Calcitriol did not halt or slow the progression of β-cell destruction; however, it reduced insulin requirement at three and six months but not at one year from the time of initiation of treatment. Another case-control pilot study evaluated the effect of 0.25µg 1-alpha-hydroxyvitamin D_3 (vitamin D analog), given twice daily to 17 patients with adult-onset latent autoimmune diabetes (LADA), on residual pancreatic β-cell function (Li et al., 2009). After one year of treatment, preserved β-cell function was only observed in patients with less than one year duration of the disease. In summary, there is

accumulating evidence that vitamin D sufficiency may play a protective role early in the pathogenesis of T1DM, but causation has not been established.

Vitamin D Status and Glucose Metabolism

Another intriguing role of vitamin D is its effect on glucose metabolism via increased insulin secretion and increased insulin sensitivity. In the 1980s, Norman and his colleagues showed the beneficial effect of vitamin D repletion on insulin secretion in vitamin D-deficient rats (Norman et al., 1980). In the 1990s, Sergeev and Rhoten found that vitamin D increases intracellular free Ca^{2+} concentration which subsequently increases insulin secretion in pancreatic β-cells (Sergeev & Rhoten, 1995). Furthermore, animal studies have shown an increased activity of 1-alpha hydroxylase in pancreatic β-cells, which suggest a possible autocrine effect of vitamin D on insulin secretion (Schwartz et al., 2004). Also, it has been suggested that vitamin D may preserve pancreatic β-cells through its effect on cell proliferation, differentiation, and apoptosis (Dusso, Brown, & Slatopolsky, 2005).

There is increasing evidence to support the link between vitamin D deficiency and altered glucose metabolism in elderly and in adults. In a cross-sectional analysis of 142 non-diabetic elderly 70-88 years in the Netherlands, vitamin D status was inversely associated with 1-hour glucose concentration ($r=-0.23$, $P<0.01$), area under the glucose curve ($r=-0.26$, $P<0.01$), and total insulin concentration ($r=-0.18$ to -0.23, $P<0.05$) during a standard 75-g oral glucose tolerance test (Baynes, Boucher, Feskens, & Kromhout, 1997). Another cross-sectional study including 126 adults with impaired glucose tolerance residing in California found a positive association between vitamin D status and insulin sensitivity index ($r=0.46$, $P=0.0007$) and a negative association between vitamin

D status and plasma glucose concentration at 60 min (r=-0.28, P=0.0011), 90 min (r=-0.28, P=0.0011), and 120 min (r=-0.29, P=0.0007) of the oral glucose tolerance test using bivariate analysis (Chiu, Chu, Go, & Saad, 2004). Furthermore, data from the NHANES (1988–1994) including 6,228 adults (2,766 non-Hispanic Whites, 1,736 non-Hispanic Blacks, and 1,726 Mexican Americans) showed an inverse association between vitamin D status and both insulin resistance and fasting glucose level in non-Hispanic Whites and Mexicans but not in non-Hispanic Blacks (Scragg, Sowers, Bell, & Third National Health and Nutrition Examination Survey, 2004).

The relationship between vitamin D status and glucose metabolism has also been examined in children and adolescents. In a cross-sectional study of 1,745 obese and Caucasian children and adolescents residing in Québec, hypovitaminosis D (25-hydroxyvitamin D <75nmol/L) was found in more than 93.0% of boys and girls. Furthermore, there was a 0.5% (P=0.015) and 0.4% (P=0.025) decrease in fasting glucose level with every 10nmol/L increase in serum 25-hydroxyvitamin D in boys and girls, respectively (Delvin et al., 2010). In a similar cohort of 127 obese children and adolescents (13.0 ± 3.0 years) residing in the Wisconsin, 25-hydroxyvitamin D was inversely associated with HbA1c (r=− 0.23, P=0.01). However, when this relationship was examined after stratifying patients by ethnicity; 25-hydroxyvitamin D remained significantly and inversely associated with HbA1c for Caucasians (r=−0.31; P=0.05), showed a trend toward significance for Hispanics (r=−0.28; P=0.085), but was not significant for African Americans (r=0.13; P=0.43) (Alemzadeh, Kichler, Babar, & Calhoun, 2008). Similarly, in a retrospective study of 302 non-diabetic children and adolescents (63.6% females; 72.5% Caucasian; 65.2% had 25-hydroxyvitamin D <

75nmol/L) residing in Minnesota, 25-hydroxyvitamin D was significantly and inversely associated with fasting glucose (r=–0.20, P<0.001) (Johnson et al., 2010).

Vitamin D Status and Immune Modulation in Vitro and in Vivo

The immune modulatory role of vitamin D was first recognized upon the identification of the vitamin D receptor in human peripheral blood monocytes and activated T cells (Bhalla, Amento, Clemens, Holick, & Krane, 1983). To date, the vitamin D receptor has been identified in various cells of the immune system including antigen presenting cells, dendritic cells, activated T and B cells, monocytes, and macrophages (Mahon, Wittke, Weaver, & Cantorna, 2003; van Etten, Stoffels, Gysemans, Mathieu, & Overbergh, 2008; Veldman, Cantorna, & DeLuca, 2000). Also, 1-α hydroxylase (CYP27B1), the enzyme that converts 25-hydroxyvitamin D to its biologically active metabolite $1,25(OH)_2D$, is expressed in activated dendritic cells, macrophages, and T and B cells (Chen et al., 2007; Hewison et al., 2007; Overbergh et al., 2000). At later stages of immune activation, 1-α hydroxylase is up-regulated and the local production of $1,25(OH)_2D$ is increased, thus creating a late negative feedback loop that down-regulates immune responses and subsequently down-regulates vitamin D receptor expression on macrophages and dendritic cells (Fritsche, Mondal, Ehrnsperger, Andreesen, & Kreutz, 2003; Kreutz et al., 1993). Furthermore, 24-hydroxylase, the enzyme that converts $1,25(OH)_2D$ to its inactive form $24,25(OH)_2D$ is expressed in immune cells and its expression is highly regulated (Chen et al., 2007; Vidal, Ramana, & Dusso, 2002).

At the cellular level, the arrangement of the $1,25(OH)_2D$/vitamin D receptor complex initiates a series of intracellular signaling events, recruits an RXR dimerization

partner, and then binds directly to intracellular vitamin D responsive elements in the promoter region of $1,25(OH)_2D$ target genes (Haussler et al., 1998; Schrader, Kahlen, & Carlberg, 1997). Of note, previously reported immune-related $1,25(OH)_2D$ target genes include cytokine genes TNFα and IFNγ (Hakim & Bar-Shavit, 2003). In essence, the 1,25(OH)2D/vitamin D receptor complex indirectly impedes the activation of immune-related transcription factors, nuclear factor of activated T cells, NFκB, and activating protein-1, thereby decreasing the production of a series of cytokines including IL-8, IL-12, IL-2, and IL-4 (Alroy et al., 1995; D'Ambrosio et al., 1998; Harant et al., 1998; Staeva-Vieira & Freedman, 2002; Takeuchi et al., 1998; Towers et al., 1999; Yu et al., 1995).

In vitro, $1,25(OH)_2D$ exerts its effect on CD4+ T cells both directly and indirectly via the regulation of dendritic cells. In the absence of antigen presenting cells, $1,25(OH)_2D$ directly inhibits CD4+ T cells differentiation towards Th17, Th1, and T follicular helper phenotypes as observed by the decreased production of IL-17, IFNγ, and IL-21, respectively (Jeffery et al., 2009). Furthermore, $1,25(OH)_2D$ increases the expression of CTLA-4 and FoxP3 and enhances the production of IL-10, thus differentiating CD4+ T cells into regulatory T cells. On the other hand, $1,25(OH)_2D$ modulates immature DCs differentiation, maturation and activation, down-regulates the expression of MHC class II, co-stimulatory molecules (CD40, CD80, CD86) and IL-12, and enhances the production of anti-inflammatory cytokines including IL-10, thereby inhibiting dendritic cell-dependent T cells activation *in vitro* (Bouillon et al., 2008; van Etten & Mathieu, 2005). 1,25(OH)2D-treated DCs become tolerogenic, give way to the induction of regulatory T cells and therefore inhibit CD4+ T cells activation and

proliferation (D'Ambrosio et al., 1998; Penna-Martinez et al., 2009). Furthermore, both the down-regulation of 1L-12 and up-regulation of IL-10 alter the cytokine milieu and shift the differentiation of CD4+ T cells towards a Th2 phenotype, thus inhibiting the production of Th1 cytokines (IFN- γ, TNF- α and IL-2) while promoting the production of Th2 cytokines IL-4, IL-5, and IL-10 (Boonstra et al., 2001; Bouillon et al., 2008).

Recently, an *in vitro* study by Giulietti and colleagues examined the anti-inflammatory role of $1,25(OH)_2D_3$ on the expression of inflammatory cytokines by freshly isolated monocytes from patients with T2DM, T1DM and healthy controls. Monocytes from individuals with T2DM ($P<0.01$) and T1DM ($P<0.05$) expressed higher levels of pro-inflammatory cytokines TNF-α, IL-6, IL-1, IL-8, COX-2, ICAM-1, and B7-1 and similar levels of anti-inflammatory cytokine IL-10 compared to monocytes from healthy controls. Furthermore, the addition of $1,25(OH)_2D_3$ to activated monocytes, down-regulated the expression of TNF-α, IL-6, IL-1, and IL-8 in monocytes from T2DM and T1DM. However, only the down-regulation of IL-8 in monocytes from T1DM reached statistical significance ($P<0.05$). $1,25(OH)_2D_3$ had no effect on IL-10 expression in either group.

In vivo, the immune modulatory effect of $1,25(OH)_2D$ on CD4+ T cells has been observed in animal models of autoimmune diseases including T1DM, systemic lupus erythematosis, experimental autoimmune encephalomyelitis, and inflammatory bowel disease (Bouillon et al., 2008; Mathieu et al., 1994; Zella, McCary, & DeLuca, 2003). Also, populations-based observational studies reported an increased incidence of multiple sclerosis, T1DM, rheumatoid arthritis, and inflammatory bowel disease in Canada, the northern parts of the United States, and Europe compared to the incidence of these

autoimmune diseases in countries closer to the equator (Cantorna & Mahon, 2004; Zipitis & Akobeng, 2008). Scientists have hypothesized that the availability of vitamin D in the environment through either sunlight or food sources is an environmental factor that may affect the prevalence of autoimmune diseases. Furthermore, individuals with autoimmune diseases were found to have genetic polymorphisms for vitamin D regulatory genes, which may increase their susceptibility to multiple sclerosis, T1DM, rheumatoid arthritis, and inflammatory bowel disease (Cantorna & Mahon, 2004).

In humans, the immune modulatory effect of vitamin D has been examined in patients with inflammatory conditions including end-stage renal disease, in elderly women post hip-fracture, in patients with chronic kidney disease, and in patients with congestive heart failure. A prospective study examined the effect of 25-hydroxyvitamin D repletion on serum inflammatory cytokine levels in seven patients with end-stage renal disease. After eight weeks of cholecalciferol therapy (50,000 U twice per week) an increase in mean serum 25-hydroxyvitamin D level from 35±5.5 to 135±8.2nmol/L was associated with a 55.0%, 30.0%, and 60.0% decrease in serum IL-8, IL-6, and TNF-α levels, respectively (Stubbs et al., 2010). Another prospective study found a significantly negative association (P=0.02) between vitamin D deficiency (25-hydroxyvitamin D <37nmol/L) and systemic levels of pro-inflammatory cytokine IL-6 in a cohort of elderly women in the year following hip-fracture (Miller et al., 2007). Interestingly, Neves and colleagues tested the effect of oral vitamin D (alfacalcidol or calcitriol-daily dose ranged from 0.25 to 1.0lg with a median of 0.5lg) on systemic levels of C-reactive protein and pro-inflammatory cytokine IL-6 in a follow up cohort of 95 patients with stages four and five chronic kidney disease. After approximately 2 years, patients who did not receive

vitamin D had higher levels of C-reactive protein (P=0.014) and a trend to higher IL-6 levels (P=0.077) compared to patients who did (Neves et al., 2010). Furthermore, a double-blind, randomized controlled trial showed that daily oral vitamin D (50 μg vitamin D_3 plus 500 mg Calcium) taken for 9 months, significantly increased systemic levels of anti-inflammatory cytokine IL-10 (P=0.042) in patients with congestive heart failure as compared to healthy controls (Schleithoff et al., 2006).

CHAPTER THREE: RESEARCH METHODS

Research Design and Target Population

The purpose of this study was to examine the relationship between 25-hydroxyvitamin D and HbA1c; and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. A cross-sectional design was used to examine these relationships in a convenience sample of children and adolescents with T1DM recruited from the Diabetes Center for Children (DCC) at the Children's Hospital of Philadelphia (CHOP). Children and adolescents were recruited based on the following inclusion and exclusion criteria.

Inclusion Criteria

1) Confirmed clinical diagnosis of T1DM
2) Age between 7 and 18 years
3) Disease duration of more than one year
4) Ability and willingness of the parents or guardian to grant consent
5) Ability of children and adolescents to grant assent

Exclusion Criteria

1) Smoking
2) Mean HbA1c >12.0% over the past year
3) Any episode of ketoacidosis within the past month
4) Presence of an inflammatory disorder (e.g. rheumatoid arthritis, inflammatory bowel disease)
5) Abnormal complete blood count, liver, or renal function
6) Any type of cancer

7) Malabsorption
8) Celiac disease
9) Current infections
10) Recent surgery
11) Use of oral steroid therapy, anti-inflammatory drugs, immunosuppressive drugs, metformin, ACE inhibitors, angiotensin II inhibitors, and/or aspirin within the past month
12) Female patients will be excluded if pregnant

Children and adolescents with less than one year duration of T1DM were excluded from this study since they may experience partial remission and thus may exhibit a different immunologic profile as compared to patients with more than one year duration of the disease (Muhammad, Swift, Raymond, & Botha, 1999). Exclusion criteria ensured that all participating children and adolescents were metabolically stable and had no co-morbid conditions other than T1DM that may affect HbA1c values or systemic levels of pro- and anti-inflammatory cytokines.

Sample Size and Power

The relationship between 25-hydroxyvitamin D (primary independent variable) and HbA1c (primary dependent variable) has not been previously examined as a primary endpoint in children and adolescents with T1DM. Therefore, to determine sample size:

1. I specified the smallest clinically meaningful change in HbA1c (%) as a function of 25-hydroxyvitamin D (nmol/L). As such, a 10nmol/L increase in 25-hydroxyvitamin D may correspond to a 0.5% decrease in HbA1c. From the SEARCH for diabetes in Youth Study (Petitti et al., 2009), mean HbA1c, in 3,947

youth with T1DM residing in the U.S., was 8.5±1.7%. Therefore, the standardized effect size was calculated by dividing 0.05 by the standard deviation 1.7, which yielded a value of 0.03.

2. I specified the type I error rate at 5.0%, which is the probability of rejecting the null hypothesis when it is actually true. I also specified the type II error rate at 20.0%, which is the probability of failing to reject the null hypothesis when it is in fact not true.
3. I selected a cross-sectional design to examine the relationship between 25-hydroxyvitamin D and HbA1c while controlling for 14 other covariates. Based on literature review, I have estimated that selected covariates explain approximately 30.0% of the variance in HbA1c. However, to be conservative, I ran sample size calculation based on a series of values corresponding to variance in HbA1c as explained by 14 selected covariates ranging from 20.0% to 50.0% (table 3.1).

Table 3.1 Power analyses and sample size calculation one

	Effect Size of Tested IV on DV	Alpha	Power	R-Squared of 14 Controlled IVs	Sample Size
1	0.03	0.05	80.0%	0.20	204
2	0.03	0.05	80.0%	0.30	178
3	0.03	0.05	80.0%	0.40	152
4	0.03	0.05	80.0%	0.50	126

Independent variable (IV); Dependent variable (DV)

From the table 3.1, I concluded that a sample size of 204 (178, 152, or 126) achieves 80.0% power to detect an R-Squared of 0.03 attributed to one independent

variable, 25-hydroxyvitamin D (nmol/L), using an F-Test with a significance level of 0.05. The variables tested were adjusted for an additional 14 independent variables with an R-Squared of 0.20 (0.30, 0.40, or 0.50).

Alternatively, Alemzadeh et al. (2008) found an inverse and significant association between 25-hydroxyvitamin D and HbA1c (r=− 0.23, P=0.01) in a cohort of 127 obese children and adolescents 13.0±3.0 years residing in Wisconsin (Alemzadeh, Kichler, Babar, & Calhoun, 2008). Accordingly, I used a correlation of −0.2 (which corresponds to an R-Squared of 0.04) to calculate the sample size for this study (table 3.2).

Table 3.2 Power analysis and sample size calculation two

	Effect Size of Tested IV on DV	Alpha	Power	R-Squared of 14 Controlled IVs	Sample Size
5	0.04	0.05	80.0%	0.20	152

Independent variable (IV); Dependent variable (DV)

Table 3.2 showed that a sample size of 152 achieves 80.0% power to detect an R-Squared of 0.04 attributed to one independent variable, 25-hydroxyvitamin D (nmol/L), using an F-Test with a significance level of 0.05. As before, the variables tested were adjusted for an additional 14 independent variables with an R-Squared equal to 0.20.

I have selected the most conservative estimates of both the R-Squared (0.03) and variance in HbA1c that is explained by 14 selected covariates (20.0%). Therefore, a sample size of 204 was considered sufficient to detect a clinically and statistically meaningful change in HbA1c as a function of 25-hydroxyvitamin D while adjusting for 14 other covariates.

Access to Target Population

Terri Lipman, PhD, CRNP, FAAN, my dissertation chair, has a clinical appointment at the Division of Endocrinology at CHOP. Dr. Lipman has granted me a non-traditional personnel (NTP) status to access my patient population and conduct my doctoral research at the DCC at CHOP. The NTP status provided me with access to CHOP IRB, facilities, intranet, patients' records (EPIC), DCC clinic schedule, and training in human subject protection including HIPAA (http://www.citiprogram.org).

Case Ascertainment

On average, children and adolescents with T1DM follow up with their diabetes provider at the DCC clinic three to four times a year. For this study, children and adolescents were recruited during their routine clinic visit at the DCC. The initial phase of recruitment entailed identifying potential participants to be informed about the study. One day prior to the clinic visit, I reviewed the clinic schedule and identified potential participants based on pre-specified inclusion and exclusion criteria. On the day of clinic visit, all potential participants had their height measured using a stadiometer (Holtain, Crymych, UK), their weight measured using a digital scale (Scale-Tronix, White Plains, New York, USA), and their HbA1c measured using point-of-care HbA1c testing (DCA Vantage, Siemens diagnostics, Tarrytown, NY). Only potential participants with HbA1c less than 12.0% and their parents were informed about the study. The following was the recruitment script:

Hello, my name is Sarah Al Sawah. I am a 4th year doctoral student at the University of Pennsylvania, School of Nursing. I am conducting a study on vitamin D status and glucose control in children and adolescents with type 1 diabetes. My mentor is Dr. Terri Lipman who is also a nurse practitioner at the DCC at CHOP.

Question 1: Is this a good time to talk to you to tell you more about the study?

You are being invited to take part in this research study because you have had type 1 diabetes mellitus for more than one year, your age is between 7 and 18 years, and your HbA1c is less than 12.0%. Your participation in this study is completely voluntary. This means that you do not have to participate in this study unless you want to. If there is anything you do not understand, please ask questions.

Question 2: Would you be interested to hear more about this study?

Good. The purpose of this research study is to find out whether vitamin D level is related to blood glucose level in children and adolescents with type 1 diabetes mellitus. Previous studies have shown that keeping vitamin D levels within the normal range is important for bone growth and development, and may be helpful in blood glucose control. The study will also look at how inflammation affects the relationship between vitamin D level and blood glucose level in young people with type 1 diabetes.

We estimate that approximately 204 subjects will enroll in this study. If you agree to take part in this study, the following procedures will be performed:

1. *We will review your medical record to collect demographic information (e.g. age, sex, ethnicity, and insurance) as well as information about your diabetes.*

2. *We will collect two teaspoons of your blood to measure vitamin D level and markers of inflammation. If you are due for your routine yearly blood tests and blood is being drawn at CHOP today, you will NOT need an extra needle stick for the two teaspoons of blood that will be collected for this study. If you go to an outside lab for blood drawing or if you are not due for your routine yearly blood tests you may still participate in the study. However, this would require an extra needle stick for the two teaspoon of blood in the CHOP laboratory.*

Your Vitamin D levels will be shared with your diabetes provider. If the results are abnormal, your diabetes provider may check your vitamin D level one more time at a local laboratory. Your participation will end once you give two teaspoons of blood.

What are the risks of this study?

Taking part in a research study involves inconveniences and risks. Taking blood may cause some pain, bleeding or bruising at the spot where the needle enters your body. Rarely, taking blood may cause fainting or infection. We will take your blood at the same time you are having blood drawn for clinical purposes. The amount of blood we will take is minimal and you will not need a separate needle stick. If you go to an outside lab for blood draw and you wish to participate in this study, you will have to undergo an additional blood draw procedure at CHOP laboratory. The risks of taking blood are the same as stated above.

As with any study involving collection of data, there is the possibility of loss of confidentiality of data. Every precaution will be taken to secure participants' personal information to ensure confidentiality.

Are there any benefits to taking part in this study?

There will be no direct benefit to the management of your glycemic control from taking part in this study. We hope that the information learned from this study will help us understand more

about type 1diabetes and possibly benefit other children and adolescents with the same condition in the future. If the results of the study show that vitamin D is related to glycemic control and blood glucose levels, the results may encourage diabetes providers to include vitamin D in yearly screening tests.

The direct benefit will come from testing your vitamin D level and providing vitamin D supplements if your vitamin D levels were low.

Financial Considerations
You will not be responsible for any costs of this study. The School of Nursing at the University of Pennsylvania and the Pediatric Endocrinology Nursing Society will pay for all costs of participating. You will be given a $5 gift card as a token of appreciation for taking part in this study.

The informed consent was signed and HIPAA authorization was obtained once potential participants and their parents expressed willingness to be part of this study (Appendix). At the end of the clinic visit, study subjects and their parents were escorted to the local laboratory at CHOP for blood drawing procedure. A non-fasting 10ml blood sample was collected in two 5ml-serum separator tubes (SSTs) from each study subject. Furthermore, each study subject received a $5 CVS gift card as a token of appreciation for participating in this study.

Sample Processing and Measurements

Once the 10ml blood sample was collected, the two 5ml-SSTs were labeled, gently mixed by inversion five times (to mix the clot activator with the blood), placed in a vertical position for 30 minutes at room temperature to allow for blood clotting, and then transported to the Biobehavioral Research Lab at the University of Pennsylvania, School of Nursing. At the Biobehavioral Research Lab, the SSTs were centrifuged at 3,000 rpm for 15 minutes to allow the separation of serum from the cellular components of blood. For each study subject, 75ul of serum was aliquoted into an eppendorf tube to instantly measure blood glucose. The remaining serum was then aliquoted into cryogenic vials and stored at −70°C until the time of batch analysis. Each study subject had four

cryogenic vials with 500ul serum in each to measure 25-hydroxyvitamin D, IL-6, IL-8, and IL-10 and a fifth cryogenic vial was labeled extra to be used if needed. All measurements were performed in duplicates to ensure reproducibility (reliability) and averages of duplicate measures were used in the final analyses (Lachin, 2004). Any extra or remaining serum will be discarded three months after publication.

Measurement of Blood Glucose

I measured blood glucose levels by glucose oxidase method using YSI 2300 STAT Blood Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA) at the Biobehavioral Research Laboratory. Glucose oxidase is an enzyme that breaks down glucose in the presence of oxygen into hydrogen peroxide and gluconic acid. Hydrogen peroxide is then oxidized as it gets in contact with a platinum electrode, producing electrons. The resultant current is then measured and is proportional to glucose concentration in the sample. The YSI 2300 STAT Blood Glucose Analyzer can detect glucose concentration as high as 900mg/dL (9000mg/L, 50.0mmol/L) with a precision of ±2.0%. YSI 2300 was calibrated at the start of every day and after every six samples. Each glucose reading required 25µl of serum and was measured in duplicate with the mean value used for statistical analyses.

Measurement of 25-hydroxyvitamin D

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) was used to measure serum concentration of 25-hydroxyvitamin D (Eisman, Shepard, & DeLuca, 1977). LC-MS/MS accurately distinguishes 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. Serum 25-hydroxyvitamin D_3 reflects endogenous cutaneous production, while serum 25-hydroxyvitamin D_2 reflects mostly dietary intake and vitamin

D supplements (Hollis, 2008). ZRT Laboratory; a for-profit corporation having a principal place of business at 8605 SW Creekside Place, Beaverton, OR 97008 ("ZRT"), was the site for 25-hydroxyvitamin D measurement. ZRT follows the Clinical Laboratory Improvement Amendments (CLIA) instituted by Centers for Medicare and Medicaid Services (CMS). ZRT LC-MS/MS assay generates results comparable with DiaSorin RIA, which has been used in major studies (Bertone-Johnson et al., 2005; Bischoff-Ferrari, Dietrich, Orav, & Dawson-Hughes, 2004; Chonchol & Scragg, 2007; Feskanich et al., 2004; Giovannucci, Liu, Hollis, & Rimm, 2008), thus providing clinically accurate and comparable results for this study. Serum samples to measure 25-hydroxyvitamin D were shipped on dry ice from the Biobehavioral Research Laboratory to ZRT using UPS 2nd Day Air.

Measurement of Cytokines

I measured pro-inflammatory cytokines IL-6 and IL-8, and anti-inflammatory cytokine IL-10 using R&D Systems ELISA assays (Minneapolis, MN) at the Biobehavioral Research Laboratory (table 3.3). All three cytokines were measured in duplicates with the mean value used for statistical analyses. Only IL-6 and IL-10 were detected in serum of study subjects. The R&D CXCL8/IL-8 ELISA assay did not detect any levels of IL-8 in our study sample.

Table 3.3 Inflammatory cytokines ELISA assays

	Mean Recovery (Range)	Intra-Assay Precision CV%	Inter-Assay Precision CV%
HS IL-6 PHS600B	94.0% (87.0-99.0%)	6.9%, 7.8%, 7.4%	9.6%, 7.2%, 6.5%
HS IL-8 S8000C	98.0% (88.0-106.0%)	5.6%, 5.4%, 6.5%	7.4%, 9.7%, 6.1%
HS IL-10 PHS100B	99.0% (88.0-112.0%)	8.2%, 8.5%, 6.6%	15.6%, 10.2%, 8.1%

Coefficient of Variation (CV)

Measurement of HbA1c

HbA1c is the gold standard index of glycemic control. It reflects mean blood glucose level over the preceding two to three months (Saudek et al., 2006). Point-of-care HbA1c testing (DCA Vantage, Siemens diagnostics, Tarrytown, NY) which is a 6-minutes test that requires approximately 1μL of whole blood sample (taken from a finger prick) was used to quantify HbA1c at the DCC. DCA Vantage meets the criteria of the National Glycohemoglobin Standardization Program (NGSP) for clinical accuracy (CV <3%) and therefore it provides a relatively accurate and comparable HbA1c values with those of the DCCT (Lenters-Westra & Slingerland, 2010; Bode, Irvin, Pierce, Allen, & Clark, 2007; K. L. Schwartz, Monsur, Hammad, Bartoces, & Neale, 2009). The DCCT used a laboratory-based ion-exchange high-performance liquid chromatography method to quantify HbA1c (Rohlfing, Wiedmeyer, Little, England, Tennill, & Goldstein, 2002).

Medical Record Review

Electronic medical records were reviewed to collect data on socio-demographic covariates (age, sex, ethnicity and insurance status), and disease-related covariates (duration of T1DM, average total daily insulin dose, type of insulin regimen, frequency of blood glucose monitoring, frequency of clinic visit, and BMI z-score) that have been updated during the clinic visit.

Selection of Covariates

By definition, a covariate is a variable related to the dependent variable that typically has a minimal relation to the independent variable (MacKinnon; 2008). For the purpose of this study, selection of covariates was based on an extensive search of the literature on predictors of and factors related to HbA1c in children and adolescents with

T1DM. Only covariates with moderate or strong associations with HbA1c were selected and were adjusted for in the multivariate analysis model. These covariates include age, sex, ethnicity, socio-economic status, duration of T1DM, average total daily insulin dose, type of insulin regimen, frequency of blood glucose monitoring, frequency of clinic visit, blood glucose level, and BMI z-score (DCCT/EDIC Research Group et al., 2009; Helgeson et al., 2010; Paris et al., 2009; Petitti et al., 2009; Rewers et al., 2002; Rohlfing, Wiedmeyer, Little, England, Tennill, & Goldstein, 2002). The Centers for Disease Control and Prevention (CDC) growth charts were used to calculate age- and sex-specific BMI z-scores; which is the "deviation of the value for an individual from the mean value of the reference population divided by the standard deviation for the reference population", to facilitate comparisons across age groups (Kuczmarski et al., 2000). Of note, insurance was selected as a proxy for socio-economic status because of the absence of alternative direct measures of socio-economic status in this study. Furthermore, insurance per se has been associated with glycemic control in adult patients with T1DM (Chew et al., 2008; Gallegos-Macias et al., 2003; Sosa-Rubi, Galarraga, & Lopez-Ridaura, 2009).

Few of the selected covariates were defined as confounders because they are related to both HbA1c (dependent variable) and 25-hydroxyvitamin D (independent variable). Confounders include age, sex, ethnicity, and BMI z-score as they explain a significant percent of variance in 25-hydroxyvitamin D and thus may change the relationship between the independent variable and dependent variable (Compher et al., 2008; Jacques et al., 1997; Macdonald et al., 2008; Yetley, 2008). Season which is a significant predictor of 25-hydroxyvitamin D but not of HbA1c was added to the list of

covariates and was categorized as spring (March–May), summer (June–August), fall (September–November), and winter (December–February). For this study, only winter, spring and summer were used since data collection took place from January till June 2011. In addition, covariates such as age, sex, ethnicity, BMI z-score, disease duration, type of insulin regimen, average total daily insulin dose, and blood glucose level are confounders that explain a significant percent of variance in both systemic levels of pro- and anti-inflammatory cytokines (hypothesized Mediators) and HbA1c (dependent variable) (Al-Isa, Thalib, & Akanji, 2010; Rosa et al., 2008; Schaumberg et al., 2005). All confounders were included as covariates in the multivariate analysis model (multiple regression analysis).

Data Management

All resultant data from 25-hydroxyvitamin D, cytokines, and blood glucose measurements were in digital printout forms (primary source documents). Also, data on covariates, which was retrieved from medical records, were recorded manually on a special study form "chart abstraction tool" (primary source document).

All data collected from medical record review and/or generated from the aforementioned laboratory assays, were coded and then entered manually into an electronic database. Each study participant was assigned a unique subject identifier that had no meaning external to this study. Personal identifiers are kept in a separate Excel sheet restricted to authorized personnel only. The electronic database is being backed-up daily. Backup CDs with data and primary source documents are stored separately in a securely locked storage facility and personal storage cabinet. The personal identifiers

excel sheet and primary source documents will be destroyed three months after publication. The electronic database will be retained for three years.

Refusal Rate and Final Study Sample

Patients were recruited between January 7, 2011 and June 17, 2011. A total of 400 children and adolescents met the inclusion and exclusion criteria and were informed about the study. 206 of the 400 children and adolescents were recruited. The majority of patients were recruited during winter (40.0%, December to February) and spring (52.0%, March to May) and the remaining were recruited early summer (8.0%, June to August).

Refusal rate was close to 48.5% which may be related to the blood drawing procedure required for participation in this study. Based on previously reported literature, refusal rate of children and families participating in clinical research have ranged from 6.7% to 45.8% for studies that did not involve blood collection (Gattuso et al., 2005). It is therefore expected that refusal rates may increase for studies involving blood collection, which is evident by the relatively high refusal rate reported for this study.

Another reason may be the fact that diabetes providers were ordering vitamin D tests on their patients more frequently at the end of the study (Mid-April through June) than at the beginning of the study (January through mid-April). Patients who already had their vitamin D test ordered as part of their annual screening did not agree to give additional blood sample for the measurement of inflammatory cytokines and therefore did not participate in this study. I was able to recruit 153 subjects January through mid-April but only 53 subjects mid-April through June.

Nine of the 206 subjects were excluded from the final analyses due to HbA1c values greater than 12.0% over the past year, coexistence of another autoimmune

condition, or presence of ketoacidosis. HbA1c values greater than 12.0% is an exclusion criteria. Six subjects with HbA1c values greater than 12.0% were mistakenly recruited due to improper documentation of their HbA1c by clinic staff or failure of PI to verify HbA1c values before recruiting subjects. One subject had celiac disease which is an exclusion criterion and was mistakenly recruited by the PI due to improper revision of the medical record. Two subjects had ketoacidosis two weeks prior to their clinic visit. Information about their ketoacidosis was documented after subjects were recruited. The final sample included 197 subjects.

Missing Data Points from Final Sample (n = 197)

Data for five variables [HbA1c, FBGM, blood glucose level, IL-6, and IL-10] were missing completely at random. HbA1c values were missing for two subjects because of insurance restrictions to perform the test at CHOP and subjects either failed to perform the test outside CHOP or failed to report results to their diabetes provider when the test was performed outside CHOP. For 29 patients, the FBGM was not documented by the diabetes provider in their medical records. One patient had a glucose level of 2.6mg/dl which was most likely a measurement error and therefore this data point was excluded from the analysis. Four patients already had their vitamin D levels ordered by their diabetes provider as part of their annual screening and have agreed to participate in the study without giving an additional blood sample for measurement of inflammatory cytokines (IL-6, IL-8, and IL-10) and blood glucose level. For two patients IL-10 was not measured due to unavailability of additional IL-10 ELISA kit to perform the measurement. Table 3.4 summarizes the frequency of missing data. FBGM has the highest percent of missing data (14.7%).

Table 3.4 Percent of missing values from final sample

Variable	Missing Observations	Percent Missing Observations
HbA1c	2/197	1
FBGM	29/197	14.7
Blood Glucose Level	5/197	2.5
IL-6	4/197	2
IL-10	6/197	3

Handling of Missing Data

To accurately examine the hypothesized relationships among key variables as proposed in aims one through three, it was crucial to address the loss of statistical power imposed by the aforementioned missing data on multivariate analyses. As table 3.5 indicates, only 160 study subjects have complete data points (i.e. missing value equals to zero).

Table 3.5 Percent of subjects with zero, one, two, or three missing values

#missing values	Freq.	Percent	Cum.
0	160	81.22	81.22
1	32	16.24	97.46
2	1	0.51	97.97
3	4	2.03	100.00
Total	197	100.00	

Multiple imputation (Stata version 12) was selected for handling missing data since data was missing completely at random, the model used to generate the imputed values was theoretically correct and matches the models subsequently used for multivariate analyses addressing aims one through three (Allison, 2000). Furthermore, it has been suggested that multiple imputation introduces appropriate random error into the imputation process, thus increasing the probability of attaining unbiased estimates of all parameters (Allison, 2000). Multiple imputation uses the distribution of the observed data

to impute missing values M times (for this analysis five times), therefore generating M complete (imputed) data sets. The imputed data sets are then analyzed individually but identically to obtain a set of parameter estimates. Finally, these parameter estimates are pooled into a single data set to generate overall parameter estimates, variances, and confidence intervals (White et al., 2011).

To select the best approach to creating multiple imputation data sets; first the pattern of missing data was examined and was determined to be arbitrary (i.e. has no pattern). Second, I examined the type of variables with missing data; FBGM was treated as categorical while HbA1c, IL-6, IL-10, and blood glucose level were treated as continuous. Therefore, the Imputation by Chained Equation (ICE) was selected as the best approach to handle both arbitrary pattern of missing data and their type. ICE can automatically differentiate between categorical and continuous variables and therefore uses multinomial logistic regression (mlogit) for unordered categorical variables and linear regression (regress) for continuous variables in the imputation process.

Prior to running Multiple Imputation with ICE, all continuous variables to be included in the imputation model were examined for normality with normal probability plots, histograms and the Shapiro-Wilk test; which is the standard test for normality in small and medium samples. Variables were considered non-normally distributed if both the Shapiro-Wilk test was significant at $P<0.05$ and the histogram showed extreme deviation from normality. Three non-normally distributed variables IL-6, IL-10, and blood glucose level were log transformed. In addition, non-normally distributed variable 'diabetes duration in months' was categorized into three levels: 1 = 12-23 months, 2 = 24-47 months, and 3 ≥48 months, to allow for comparability of the results with

previously published SEARCH for Diabetes in Youth study (Pettiti et al., 2009). FBGM which has values 0 through 4 and greater than 4 time per day was categorized into three levels: 1 =0-2 times per day, 2 =3-4 times per day, and 3 ≥4 times per day. Frequency of nurse practitioner visit which has values zero through 6 visits over the past year was categorized into two levels: 1 =1-3 visits over the past year, and 2 =4-6 visits over the past year. This step of treating variables was necessary to ensure that the model used to generate the imputed values match with subsequent multivariate models used to examine aims one through three. The table below (table 3.6) compares the regression output of imputed versus non-imputed model. Since the imputed model is based on a sample size of 197, it provides more accurate estimates of the regression coefficients as compared to the non-imputed model which is based on a sample size of 160. Furthermore, there are no concerning differences in regression coefficients and significance levels between the two models, except for insurance status which is only significant in the imputed model (n = 197). Therefore, the imputed model was used for subsequent multivariate analyses. For descriptive statistics, the non-imputed data set was used.

Table 3.6 Comparison of regression output of imputed versus non-imputed model for HbA1c regressed on 25-hydroxyvitamin D while adjusting for select covariates

	Imputed Model 1 **(F=9.69, P=0.000) (n = 197)**	Non-imputed Model 2 **(F=8.53, P=0.000) (n = 160)**
Age at examination, years[C]	-0.016, ***0.610***	-0.007, ***0.829***
Sex[CAT]	0.026, ***0.878***	0.137, ***0.459***
Ethnicity[CAT]	0.220, ***0.035****	0.318, ***0.007****
Insurance status [CAT]	0.413, ***0.024****	0.311, ***0.121***
BMI z-score[C]	0.028, ***0.796***	-0.014, ***0.905***
Diabetes duration, months[CAT]	0.322, ***0.008****	0.330, ***0.011****
Type of Insulin Regimen[CAT]	0.261, ***0.002****	0.236, ***0.012****
Average Total Daily Insulin Dose, units/kg/day[C]	0.679, ***0.013****	0.698, ***0.013****
Frequency of Blood Glucose Monitoring per Day[CAT]	-0.588, ***0.000****	-0.553, ***0.000****
Frequency of CRNP Clinic Visit over the past Year[CAT]	-0.362, ***0.028****	-0.477, ***0.008****
25-hydroxyvitamin D, nmol/L[C]	0.007, ***0.154***	0.006, ***0.317***
Log-Mean Glucose, mg/dl[C]	0.329, ***0.018****	0.282, ***0.048****
Log-IL-6, pg/ml[C]	0.060, ***0.564***	-0.022, ***0.844***
Log-IL-10, pg/ml[C]	0.201, ***0.013****	0.250, ***0.004****

***P <0.05, ** P < 0.01**
[C], continuous variables; [CAT], categorical variables

Statistical Analyses

Step 1: Descriptive statistics were used to summarize the socio-demographic and diabetes-related characteristics of the final sample (n=197) of children and adolescents with T1DM (table 4.1). Frequencies and percentages were used to describe categorical variables. Means and standard deviations were used to describe continuous variables that were normally distributed; while medians, minima, and maxima were used to describe continuous variables that were non-normally distributed. Normality of continuous variables was assessed using normal probability plots, histograms and the Shapiro-Wilk test; which is the standard test for normality in small and medium samples. Variables were considered non-normally distributed if the Shapiro-Wilk test was significant at $P<0.05$ and the histogram showed extreme deviation from normality. SPSS version 19 was used for descriptive statistics. For the remaining analyses, Stata version 12 was used.

Step 2: Univariate associations between HbA1c (primary dependent variable; treated as continuous) and socio-demographic and diabetes-related characteristics were tested for statistical significance using general linear modeling (GLM) (table 4.2a). The sample was stratified into percentages of individuals with good, intermediate, and poor glycemic control by socio-demographic and disease-related characteristics. Mean HbA1c was calculated for each stratification.

Furthermore, the univariate associations between HbA1c and socio-demographic and diabetes-related characteristics were re-examined separately for Caucasians and African Americans (tables 4.2b and 4.2c). These associations were not examined for Hispanics or Others because of the small sample size associated with these categories (n=15 and n=8; respectively).

Step 3: Similarly, univariate associations between 25-hydroxyvitamin D (primary independent variable, treated as continuous) and socio-demographic and select diabetes-related characteristics were tested for statistical significance using GLM (tables 4.3a and 4.3b). In table 4.3a, the sample was stratified into percentages of individuals at risk of vitamin D deficiency, risk of vitamin D inadequacy, and sufficient in vitamin D--according to IOM classification of vitamin D status. In table 4.3b, the sample was stratified into percentages of individuals with vitamin D deficiency, insufficiency, and sufficiency based on recent recommendations of the Endocrine Society. Mean 25-hydroxyvtamin D was calculated for each stratification.

Step 4: GLM was also used to examine the univariate associations between inflammatory cytokines (IL-6 and IL-10) and socio-demographic and diabetes-related characteristics (table 4.4a). For sake of consistency with the multivariate models, the log transformed forms of IL-6 and IL-10 were used for univariate analyses. Otherwise, means of IL-6 and IL-10 were calculated from non-log transformed values. Furthermore, the univariate associations between inflammatory cytokines and socio-demographic and disease related characteristics were re-examined separately for Caucasians and African Americans (tables 4.4b and 4.4c).

Step 5: Pearson product-moment coefficient of correlation (r) was used to examine the extent to which select continuous variables correlate prior to their simultaneous inclusion in subsequent regression analyses. As shown in table 4.5, the correlation coefficients ranged from -0.0003 to 0.26 which were considered substantially low enough to allow the inclusion of all 8 variables simultaneously in multivariate models.

Step 6_Aim One: GLM was used to quantify the relationship between 25-hydroxyvitamin D (independent variable) and HbA1c (dependent variable), while controlling for select covariates and excluding IL-6 and IL-10 from the multivariate model (tables 4.6 and 4.7). Covariates were included in the multivariate model if: a) they were correlated to HbA1c in univariate analyses at $P<0.20$ or b) they were known a priori to correlate to HbA1c.

Step 7_Aim Two: GLM was used to quantify the relationship between 25-hydroxyvitamin D (independent variable) and IL-6 (dependent variable), while controlling for select covariates and excluding HbA1c from the multivariate model (tables 4.8). Covariates were included in the multivariate model if: a) they were correlated to IL-6 in univariate analyses at $P<0.20$ or b) they were known a priori to correlate to IL-6.

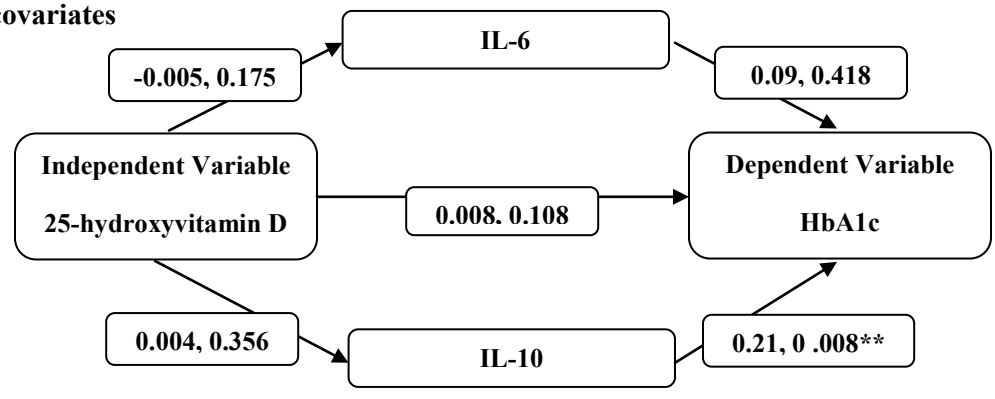
Step 8_Aim Two: GLM was used to quantify the relationship between 25-hydroxyvitamin D (independent variable) and IL-10 (dependent variable), while controlling for select covariates and excluding HbA1c from the multivariate model (table 4.9). Covariates were included in the multivariate model if: a) they were correlated to IL-10 in univariate analyses at $P<0.20$ or b) they were known a priori to correlate to IL-10.

Step 9_Aim Three: GLM was used to quantify the relationship between IL-6 (independent variable) and HbA1c (dependent variable), while controlling for select covariates and excluding 25-hydroxyvitamin D from the multivariate model (table 4.10). Covariates were included in the multivariate model if: a) they were correlated to HbA1c in univariate analyses at $P<0.20$ or b) they were known a priori to correlate to HbA1c.

Step 10_Aim Three: GLM was used to quantify the relationship between IL-10 (independent variable) and HbA1c (dependent variable), while controlling for select covariates and excluding 25-hydroxyvitamin D from the multivariate model (table 4.11). Covariates were included in the multivariate model if: a) they were correlated to HbA1c in univariate analyses at P<0.20 or b) they were known a priori to correlate to HbA1c.

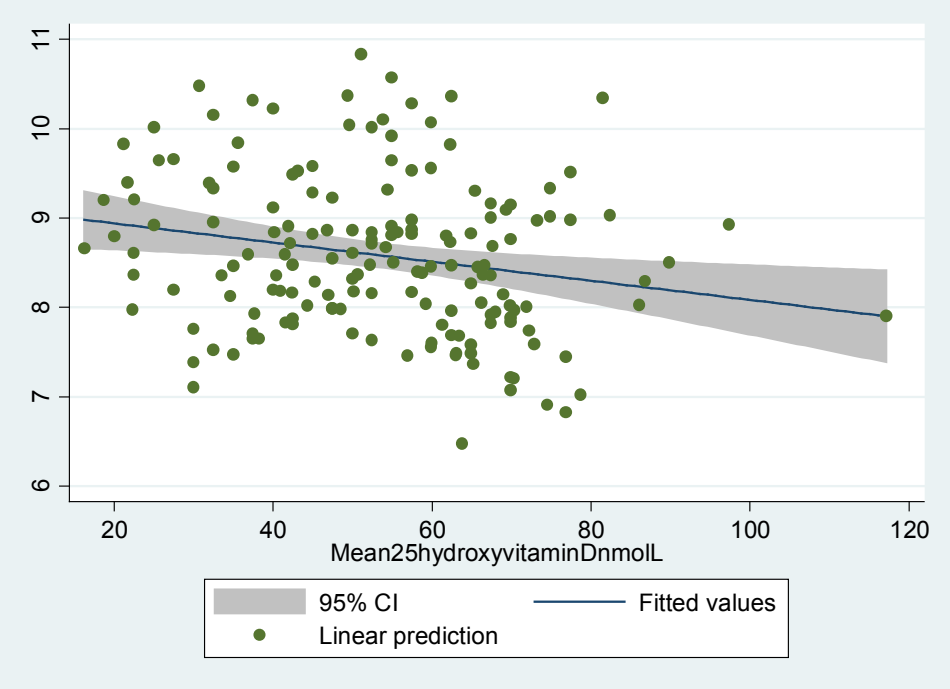
Step 11_Aim Four: Aim four was to determine whether the relationship between 25-hydroxyvitamin D and HbA1c is mediated by inflammatory cytokines IL-6, IL-8, and IL-10. Pro-inflammatory cytokine IL-8 was not detected in this sample. Furthermore, based on previous analyses there was no association between 25-hydroxyvitamin D (independent variable) and HbA1c (dependent variable). Also, there was no association between 25-hydroxyvitamin D and inflammatory cytokines IL-6 or IL-10 (mediators). The hypothesis that inflammatory cytokines IL-6, IL-8, and IL-10 mediate the relationship between 25-hydroxyvitamin D and HbA1c did not hold true in this study sample. Therefore, no further analyses to assess the significance of mediated (indirect) effect using bootstrapping were conducted.

Figure 3.1 This figure is a visual representation of the tested relationships among 25-hydroxyvitamin D, HbA1c, and inflammatory cytokines IL-6 and IL-10, adjusted for select covariates



Step 12_Aim Five: Aim five was to determine a threshold value for minimum mean 25-hydroxyvitamin D level beyond which greater improvements in HbA1c may be expected. Based on previous analyses, the relationship between 25-hydroxyvitamin D and HbA1c was found to be linear after adjusting for select covariates and therefore, it was not possible to determine a threshold value for minimum mean 25-hydroxyvitamin D beyond which greater improvements in HbA1c may exist (figure 3.2).

Figure 3.2 Two way scatter plot depicting the linear relationship between 25-hydroxyvitamin D and HbA1c



Protection of Human Subjects

Potential risk or harm for patients participating in this study was not greater than minimal. According to CHOP IRB SOP 401, minimal risk is defined as "...the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests...."

Informed Consent and HIPAA Authorization

The informed consent and HIPAA authorization were signed once patients and their parents agreed to participate in this study. The language of the informed consent was clear and comprehensive. It listed the anticipated risks and benefits associated with this study and the policy of the institution for public data sharing. Assent was obtained from all participating children and adolescents.

Risk Assessment

Risks associated with blood withdrawal procedure were mild to moderate pain at the time of blood drawing procedure. Alternately, risks due to breach of confidentiality such as public or unauthorized disclosure of personal identifiers were nonexistent.

Benefits of Study Participation

There was no direct benefit to the management of glycemic control for subjects taking part in this study. However, study subjects had their vitamin D testing at a CLIA certified laboratory free of charge. In addition, study subjects with vitamin D deficiency and insufficiency were followed up by the dietitian and were started on vitamin D supplements.

Payment to subjects and families

Each study subject received a $5 CVS gift card as a token of appreciation for participating in this study.

Time Line

The total duration of this study was close to 10 months. Data collection and measurement took a total of 6 months. Data management, data analyses and write up were 4 months.

CHAPTER FOUR: RESULTS

The aim of this study was to examine the relationship between 25-hydroxyvitamin D and HbA1; and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. A total of 206 subjects were recruited at the DCC at CHOP. Only 197 subjects were included in the final analyses.

Socio-Demographics and Diabetes-Related Characteristics of Final Sample (Table 4.1)

The mean age of the study sample was 13±3years. The slight majority of study subjects were adolescents (55.3%), males (56.9%), and Caucasian (54.8%). Other racial/ethnic groups included African Americans (33.5%), Hispanics (7.6%), and Others (4.1%). The majority of study subjects had private insurance (70.0%). 63.5% of study subjects were below the 85th percentile for age- and sex- adjusted body weight (healthy weight), 23.4% were between the 85th and 95th percentile (overweight), and 13.2% were above the 95th percentile (obese).

All study subjects were recruited after the first year of diagnosis to bypass the honeymoon phase. More than half of study subjects had T1DM for ≥48 months (57.9%), 27.4% had T1DM for 24-47 months, and 14.7% had T1DM for 12-23 months. Mean HbA1c of the study sample was 8.6±1.4% with an average total daily insulin dose of 0.87±0.3units/kg/day. Only 36.0% of study subjects received insulin replacement therapy via insulin pump, almost half of study subjects received multiple daily insulin injections (Lantus or NPH) while the remaining were taking mixed insulin twice per day (12.2%). Data on FBGM was missing for 29 study subjects. Among those who reported their

FBGM, approximately 88.0% checked their blood glucose levels ≥3 times per day. Furthermore, the slight majority of study subjects (60.9%) followed up with their nurse practitioner at least 4-6 times during the past year.

Mean 25-hyroxyvitamin D for the final cohort was 54.6±17.8nmol/L. Median blood glucose level, pro-inflammatory cytokine IL-6 level, and anti-inflammatory cytokine IL-10 level were 160mg/dl (range; 44 to 710mg/dl), 0.7pg/ml (range; 0.1 to 9.4pg/ml), and 4.5pg/ml (range, 0.6 to 152.9pg/ml), respectively. As previously reported in chapter three, systemic levels of pro-inflammatory cytokine IL-8 were not detected in this study sample.

Table 4.1 Socio-demographics and diabetes-related characteristics of final sample (n = 197)

Age at examination, years (Mean ± SD)	13 ± 3
7-12 (n, percentage)	88 (44.7)
13-18 (n, percentage)	109 (55.3)
Sex (n, percentage)	
Male	112 (56.9)
Female	85 (43.1)
Ethnicity (n, percentage)	
Caucasian	108 (54.8)
African-American	66 (33.5)
Hispanic	15 (7.6)
Other	8 (4.1)
Insurance (n, percentage)	
Private	138 (70.0)
Medicaid	59 (30.0)
BMI z-score (Mean ± SD)	0.76 ± 0.78
BMI Percentile (n, percentage)	
Healthy weight ($< 85^{th}$)	125 (63.5)
Overweight ($\geq 85^{th}$ & $< 95^{th}$)	46 (23.4)
Obese ($\geq 95^{th}$)	26 (13.2)
Diabetes Duration, months [Median (min, max)]	54 (12, 179)
12-23	29 (14.7)
24-47	54 (27.4)
≥ 48	114 (57.9)
Type of Insulin Regimen (n, percentage)	
Insulin Pump	71 (36.0)
Multiple Daily Injections, Lantus	59 (29.9)
Multiple Daily Injections, NPH	43 (21.8)
Mixed Insulin Twice per Day	24 (12.2)
Average Total Daily Insulin Dose, units/kg/day (Mean ± SD)	0.87 ± 0.3
Frequency of Blood Glucose Monitoring per Day (n, percentage)	
0-2	19 (9.6)
3-4	71 (36.0)
> 4	78 (39.6)
Missing data	29 (14.7)
Frequency of CRNP Clinic Visit over the past Year (n, percentage)	
1-3	77 (39.1)
4-6	120 (60.9)
HbA1c, % (Mean ± SD)	8.6 ± 1.4

25-hydroxyvitamin D, nmol/L (Mean ± SD)	54.6 ± 17.8
Mean Glucose, mg/dl [Median (min, max)]	160 (44, 710)
IL-6, pg/ml [Median (min, max)]	0.7 (0.1, 9.4)
IL-10, pg/ml [Median (min, max)]	4.5 (0.6, 152.9)
IL-8, pg/ml	Not Detected

Stratification of Final Sample into Good, Intermediate, and Poor Glycemic Control by Socio-Demographic and Disease-Related Characteristics (n = 197) (Table 4.2a)

The ADA has set target values for optimal HbA1c levels by age group. HbA1c levels less than 8.0% for ages 6-12 years and HbA1c levels less than 7.5% for ages 13-18 years have been classified as good control. HbA1c levels equal to or more than 9.5% have been classified as poor glycemic control for all age groups. For any given age group, HbA1c levels between good and poor glycemic control have been classified as intermediate glycemic control. Using the ADA classification for glycemic control, 27.9% of the final sample had good glycemic control, 48.2% had intermediate control, and 22.8% had poor control. Glycemic control was not statistically different for males versus females (P=0.355) or for study subjects across different BMI percentiles (P=0.396). Study subjects 13-18 years were more likely to have poor glycemic control as compared to the younger age group (P=0.084). African Americans and Hispanics were significantly more likely to have poor glycemic control as compared to Caucasians (P<0.000). Similarly, poor glycemic control was significantly more evident in subjects who had Medicaid versus subjects with private insurance (P<0.000); in subjects with ≥48 months disease duration as compared to subjects with ≤48 months disease duration (P<0.000); in subjects receiving insulin two times per day as compared to subjects receiving multiple daily injections per day (NPH and Lantus), or on insulin pump (P<0.000). Furthermore, HbA1c was significantly higher in study subjects reporting FBGM 0-2 times per day as compared to study subjects monitoring their blood glucose levels 3-4 times per day or ≥ 4 times per day (P<0.000). Also, study subjects who followed with their nurse practitioner 4-6 times during the past year were significantly more likely to have lower levels of

HbA1c as compared to study subjects who followed with their nurse practitioner 1-3 times during the past year (P=0.005). Although not statistically significant, there was a trend of decreasing levels of HbA1c as 25-hydroxyvitamin D levels improved from levels at risk of deficiency towards sufficient states (P=0.286). The bivariate relationship between HbA1c and 25-hydroxyvitamin D almost reached statistical significance (P=0.057).

Table 4.2a Stratification of Sample into Good, Intermediate, and Poor Glycemic Control by Socio-Demographic and Disease-Related Characteristics (n = 197)

		HbA1c (%)[†C]				
	N	Mean HbA1c	Good (%)	Intermediate (%)	Poor (%)	P-value
All Sample	197	8.6±1.4	27.9	48.2	22.8	
Age at examination, years [C]						***0.136***
Age at examination, years [CAT]						***0.084***
7-12	88	8.4±1.2	37.5	43.2	19.3	
13-18	109	8.7±1.5	20.2	52.3	25.7	
Sex [CAT]						***0.355***
Male	112	8.6±1.4	26.8	46.4	25.0	
Female	85	8.5±1.3	29.4	50.6	20.0	
Ethnicity [CAT]						***<0.000****
Caucasian	108	8.1±1.1	37.0	54.6	8.3	
African-American	66	9.2±1.5	15.2	39.4	42.4	
Hispanic	15	9.4±1.2	6.7	53.3	40.0	
Others	8	8.2±1.7	50.0	25.0	25.0	
Insurance status [CAT]						***<0.000****
Private	138	8.3±1.3	34.1	48.6	15.9	
Medicaid	59	9.2±1.3	13.6	47.5	39.0	
BMI z-score [C]						***0.187***
BMI Percentile [CAT]						***0.396***
Healthy weight (< 85th)	125	8.5±1.4	29.6	47.2	21.6	
Overweight (≥ 85th & < 95th)	46	8.7±1.5	26.1	47.8	26.1	
Obese (≥ 95th)	26	8.7±1.0	23.1	53.8	23.1	
Diabetes duration, months [CAT]						***<0.000****
12-23	29	8.0±1.2	44.8	37.9	13.8	
24-47	54	8.3±1.2	33.3	46.3	18.5	
≥ 48	114	8.8±1.4	21.1	51.8	27.2	
Type of Insulin Regimen [CAT]						***<0.000****
Insulin Pump	71	8.1±1.1	38.0	52.1	9.9	
MDI, Lantus	59	8.4±1.2	27.1	57.6	13.6	
MDI, NPH	43	8.7±1.4	25.6	46.5	25.6	
Mixed Insulin 2x/day	24	10.1±1.3	4.2	16.7	79.2	
Average Total Daily Insulin Dose, units/kg/day [C]						***<0.000****

Frequency of Blood Glucose Monitoring per Day [CAT]						***<0.000***
0-2	19	10.0±1.3	31.6	63.2	5.3	
3-4	71	8.9±1.4	16.9	52.1	29.6	
> 4	78	8.0±1.0	43.6	48.7	7.7	
Frequency of CRNP Clinic Visit over the past Year [CAT]						***0.005****
1-3	77	8.9±1.4	22.1	42.9	32.5	
4-6	120	8.3±1.3	31.7	51.7	16.7	
25-hydroxyvitamin D, nmol/L [C]						***0.057***
25-hydroxyvitamin D, nmol/L [CAT]						***0.286***
At risk of deficiency[£]	17	9.0±1.6	11.1	55.6	27.8	
Risk of Inadequacy[£]	62	8.5±1.3	29.0	45.2	25.8	
Sufficient-1[£]	96	8.6±1.3	28.9	47.4	22.7	
Sufficient-2[£]	20	8.2±1.4	35.0	55.0	10.0	
Log Mean Glucose, mg/dl [C]						***0.004****
Log IL-6, pg/ml [C]						***0.253***
Log IL-10, pg/ml [C]						***0.016****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between HbA1c (treated as continuous) and select variables. P-value <0.05 is considered significant.

[†]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

Stratification of Caucasians and African Americans into Good, Intermediate, and Poor Glycemic Control by Socio-Demographic and Disease-Related Characteristics (Tables 4.2b and 4.2c)

The observed trend of decreasing levels of HbA1c with increasing levels of 25-hydroxyvitamin D for the final study sample compelled further examination of this relationship by race/ethnicity. Table 4.2b re-examined univariate associations between HbA1c and socio-demographic and disease-related characteristics in Caucasians (n=108). Similar to the study sample (n=197), poor glycemic control was significantly more evident in subjects who had Medicaid versus subjects with private insurance (P=0.009), and in subjects reporting FBGM of 0-2 times per day as compared to subjects reporting FBGM of 3-4 times per day or ≥4 times per day (P<0.000). Interestingly, although not evident in the study sample (n=197), BMI z-score almost reached statistical significant as a predictor of glycemic control in Caucasians (P=0.053). Conversely, diabetes duration, type of insulin regimen, frequency of nurse practitioner clinic visit over the past year, and 25-hydroxyvitamin D did not retain statistical significance.

Table 4.2c re-examined univariate associatiosn between HbA1c and socio-demographic and disease-related characteristics in African Americans (n=66). As compared to the final study sample (n=197), diabetes duration (P=0.002), type of insulin regimen (P=0.002), and FBGM (P=0.013) were significant determinants of poor glycemic control in African Americans; while BMI z-score, insurance status, frequency of nurse practitioner clinic visit in the past year, and 25-hydroxyvitamin D were not statistically significant.

As compared to African Americans, Caucasians were more likely to have private insurance (84.25% versus 46.96%), receive insulin replacement therapy via insulin pump (45.37% versus 19.69%), report monitoring their blood glucose levels ≥4 times per day (59.34% versus 22.8%), and follow up with nurse practitioner 4-6 times during the past year (66.6% versus 54.5%). These observed differences between Caucasians and African Americans suggest that insurance status, type of insulin regimen, FBGM, and frequency of clinic visit may be important determinants of glycemic control in this study sample. However, in previous studies, it has been reported that the relationship between insurance status and glycemic control loses statistical significance when adjusting for family structure, level of parental education, and household income in multivariate analyses (Gallegos-Macias et al., 2003; Levine et al., 2001; Paris et al., 2009; Petitti et al., 2009). In this study, I did not collect information on family structure, level of parental education, or household income. Therefore, it is unclear whether those observed differences associated with insurance status are related to socio-economic status, family structure or level of parental education in this sample. In addition, the observed difference related to type of insulin regimen and frequency of clinic visit may be related to diabetes provider bias, environmental barriers, or parental choices. It can also be argued that the value of HbA1c have influenced the choice of insulin regimen. Furthermore, data on FBGM is based on self-report and therefore the observed differences related to FBGM between Caucasians and African Americans may be inaccurate. It can also be argued that the observed differences in HbA1c between Caucasians and African Americans may be inherently physiologic. Recent studies have shown that African Americans have higher HbA1c levels than Caucasians across the full spectrum of glycemia (Zlemer et al., 2010).

Table 4.2b Stratification of Caucasians into good, intermediate, and poor glycemic control by socio-demographic and disease-related characteristics (n = 108)

		HbA1c (%) [†C]				
	N	Mean HbA1c	Good (%)	Intermediate (%)	Poor (%)	P-value
Caucasians	108	8.1±1.1	37.0	54.6	8.3	
Age at examination, years [C]						***0.235***
Age at examination, years [CAT]						***0.140***
7-12	48	7.9±1.0	47.9	45.8	6.3	
13-18	60	8.3±1.1	28.3	61.7	10.0	
Sex [CAT]						***0.092***
Male	69	8.3±1.1	33.3	56.5	10.1	
Female	39	7.9±1.1	43.6	51.3	5.1	
Insurance status [CAT]						***0.009****
Private	91	8.0±1.1	42.9	50.5	6.6	
Medicaid	17	8.7±0.7	5.9	76.5	17.6	
BMI z-score [C]						***0.053***
BMI Percentile [CAT]						***0.181***
Healthy weight (< 85th)	72	8.1±1.1	40.3	51.4	8.3	
Overweight (≥ 85th & < 95th)	22	8.0±1.0	36.4	59.1	4.5	
Obese (≥ 95th)	14	8.6±1.0	21.4	64.3	14.3	
Diabetes duration, months [CAT]						***0.090***
12-23	16	7.9±1.3	62.5	25.0	12.5	
24-47	30	7.9±1.0	43.3	46.7	10.0	
≥ 48	62	8.3±1.0	27.4	66.3	6.5	
Type of Insulin Regimen [CAT]						***0.226***
Insulin Pump	49	8.0±1.1	38.8	55.1	6.1	
MDI, Lantus	39	8.1±1.0	35.9	59.0	5.1	
MDI, NPH	18	8.3±1.3	38.9	44.4	16.7	
Mixed Insulin 2x/day	2	9.1±0.7		50.0	50.0	
Average Total Daily Insulin Dose, units/kg/day [C]						***0.004****
Frequency of Blood Glucose Monitoring per Day [CAT]						***<0.000****
0-2	5	9.3±1.4		60.0	40.0	
3-4	32	8.4±1.2	25.0	62.5	12.5	
> 4	54	7.8±0.8	48.1	50.0	1.9	

Frequency of CRNP Clinic Visit over the past Year [CAT]						***0.440***
1-3	36	8.9±1.4	36.1	55.6	8.3	
4-6	72	8.3±1.3	37.5	54.2	8.3	
25-hydroxyvitamin D, nmol/L [C]						***0.966***
25-hydroxyvitamin D, nmol/L [CAT]						***0.523***
At risk of deficiency[£]	2	7.2±1.3	50.0	50.0	-	
Risk of Inadequacy[£]	31	8.0±1.1	41.9	51.6	6.5	
Sufficient-1[£]	62	8.3±1.0	33.9	54.8	11.3	
Sufficient-2[£]	13	7.9±1.2	38.5	61.5	-	
Log Mean Glucose, mg/dl [C]						***0.035****
Log IL-6, pg/ml [C]						***0.595***
Log IL-10, pg/ml [C]						***0.695***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between HbA1c (treated as continuous) and select variables. P-value <0.05 is considered significant.

[†]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

Table 4.2c Stratification of African Americans into good, intermediate, and poor glycemic control by socio-demographic and disease-related characteristics (n = 66)

		HbA1c (%) †C				
	N	Mean HbA1c	Good (%)	Intermediate (%)	Poor (%)	P-value
African Americans	66	9.2±1.5	15.2	39.4	42.4	
Age at examination, years C						***0.543***
Age at examination, years CAT						***0.785***
7-12	29	9.0±1.3	20.7	37.9	41.4	
13-18	37	9.2±1.7	10.8	40.5	43.2	
Sex CAT						***0.771***
Male	29	9.3±1.7	17.2	27.6	48.3	
Female	37	9.1±1.3	13.5	48.6	37.8	
Insurance status CAT						***0.357***
Private	31	9.0±1.5	12.9	45.2	35.5	
Medicaid	35	9.3±1.5	17.1	34.3	48.6	
BMI z-score C						***0.673***
BMI Percentile CAT						***0.979***
Healthy weight (< 85th)	40	9.1±1.4	12.5	42.5	40.0	
Overweight (≥ 85th & < 95th)	16	9.4±1.9	18.8	31.3	50.0	
Obese (≥ 95th)	10	8.9±1.1	20.0	40.0	40.0	
Diabetes duration, months CAT						***0.002*****
12-23	12	8.3±0.9	16.7	58.3	16.7	
24-47	16	8.7±1.6	25.0	37.5	31.3	
≥ 48	38	9.6±1.4	10.5	34.2	55.3	
Type of Insulin Regimen CAT						***0.002*****
Insulin Pump	13	8.6±1.3	23.1	46.2	30.8	
MDI, Lantus	14	8.7±1.3	14.3	50.0	28.6	
MDI, NPH	21	8.9±1.4	19.0	47.6	28.6	
Mixed Insulin 2x/day	18	10.1±1.5	5.6	16.7	77.8	
Average Total Daily Insulin Dose, units/kg/day C						***0.031****
Frequency of Blood Glucose Monitoring per Day CAT						***0.013****
0-2	10	10.1±1.2		20.0	70.0	
3-4	34	9.2±1.6	11.8	41.2	44.1	
> 4	13	8.5±1.3	38.5	38.5	23.1	

Frequency of CRNP Clinic Visit over the past Year [CAT]						***0.194***
1-3	30	9.5±1.5	10.0	30.0	53.3	
4-6	36	8.9±1.5	19.4	47.2	33.3	
25-hydroxyvitamin D, nmol/L [C]						***0.578***
25-hydroxyvitamin D, nmol/L [CAT]						***0.365***
At risk of deficiency[£]	14	9.2±1.6	7.1	57.1	28.6	
Risk of Inadequacy[£]	25	8.9±1.5	20.0	32.0	48.0	
Sufficient-1[£]	25	9.4±1.4	16.0	36.0	44.0	
Sufficient-2[£]	2	9.5±0.9		50.0	50.0	
Log Mean Glucose, mg/dl [C]						***0.094***
Log IL-6, pg/ml [C]						***0.655***
Log IL-10, pg/ml [C]						***0.006*****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between HbA1c (treated as continuous) and select variables. P-value <0.05 is considered significant.

[†]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

Stratification of Final Sample by Vitamin D Status (Tables 4.3a and 4.3b)

In November of 2010, the Institute of Medicine (IOM) released an update on the recommended daily allowance for calcium and vitamin D intake for the general healthy U.S. population. In this report, the IOM also redefined vitamin D status based on evidence from studies on vitamin D and calcium (IOM, 2010). According to the IOM, vitamin D status is classified into four categories: at risk of vitamin D deficiency (25-hydroxyvitamin D less than 30 nmol/L), at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L), sufficient in vitamin D (25-hydroxyvitamin D equal or more than 50 and equal or less than 125nmol/L), and possibly harmful vitamin D (25-hydroxyvitamin D more than 125nmol/L). Vitamin D levels in this study cohort ranged from 16nmol/L to 117nmol/L, and therefore none of the study subjects had possible harmful vitamin D levels. More than half of study subjects had sufficient vitamin D levels, 31.5% were at risk of vitamin D inadequacy, and only 9.1% were at risk of vitamin D deficiency (table 4.3a). The percent of study subjects at risk for vitamin D deficiency is comparable to what has been previously reported for the U.S. pediatric population (9% vitamin D deficiency defined in the NHANES study as 25-hydroxyvitamin D less than 37nmol/L) (Kumar, Muntner, Kaskel, Hailpern, & Melamed, 2009).

Vitamin D levels were significantly lower in study subjects 13-18 years as compared to the younger age group (P=0.001), in females as compared to males (P=0.034), and in African Americans as compared to Caucasians (P=0.000). Surprisingly, mean vitamin D levels were similar for subjects across different BMI levels (P=0.486), although risk of vitamin D deficiency was more evident in subjects with obesity (3.8%)

as compared to subjects who were overweight (10.9%) or healthy weight (9.6%). Also, the risk of vitamin D deficiency was higher during Winter (10.3%) as compared to Spring (8.7%) or Summer (6.3%) although the difference was not statistically significant (P=0.091). These results were expected since the majority of study subjects were recruited during Winter and Spring (91.87%).

Table 4.3a Stratification of final sample into sufficient, inadequate and deficient vitamin D status according to Institute of Medicine recommendations (n=197)

		25-hydroxyvitamin D (25-(OH)D; nmol/L) [†] [C]				
	N	Mean 25-(OH)D	Risk of Deficiency (%)	Risk of Inadequacy (%)	Sufficient (%)	P-value
All Sample	197	54.6±17.8	9.1	31.5	59.4	
Age at examination, years [C]						***0.001****
Age at examination, years [CAT]						***0.001****
7-12	88	59.3±17.3	3.4	26.1	70.5	
13-18	109	50.8±17.4	13.8	35.8	50.5	
Sex [CAT]						***0.034****
Male	112	56.9±16.7	4.5	31.3	64.3	
Female	85	51.5±18.8	15.3	31.8	52.9	
Ethnicity [CAT]						***0.195***
Caucasian	108	59.3±16.3	1.9	28.7	69.4	
African-American	66	45.0±15.6	21.2	37.9	40.9	
Hispanic	15	55.9±21.4	13.3	33.3	53.3	
Others	8	68.7±15.0	-	12.5	87.5	
Insurance status [CAT]						***0.022****
Private	138	56.5±18.0	9.4	26.8	63.8	
Medicaid	59	50.1±16.8	8.5	42.4	49.2	
BMI z-score [C]						***0.486***
BMI Percentile [CAT]						***0.696***
Healthy weight (< 85th)	125	54.8±18.9	9.6	34.4	56.0	
Overweight (≥ 85th & < 95th)	46	54.9±16.6	10.9	21.7	67.4	
Obese (≥ 95th)	26	53.0±15.1	3.8	34.6	61.5	
Type of Insulin Regimen [CAT]						***0.001***
Insulin Pump	71	58.8±19.2	7.0	25.4	67.6	
MDI, Lantus	59	54.9±16.1	5.1	37.3	57.6	
MDI, NPH	43	52.8±16.3	9.3	34.9	55.8	
Mixed Insulin 2x/day	24	44.6±17.0	25.0	29.2	45.8	
Average Total Daily Insulin Dose, units/kg/day [C]						***0.002****
Season [CAT]						***0.091***
Winter	78	53.5±17.5	10.3	34.6	55.1	
Spring	103	53.7±16.9	8.7	33.0	58.3	

Summer	16	65.5±22.3	6.3	6.3	87.5
Log Mean Glucose, mg/dl [C]					***0.195***
Log IL-6, pg/ml [C]					***0.031****
Log IL-10, pg/ml [C]					***0.730***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between 25-hydroxyvitamin D (treated as continuous) and select variables. P-value <0.05 is considered significant.

[†] Study sample was stratified into three quartiles according to the IOM classification for vitamin D status: at risk of vitamin D deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of vitamin D inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), and sufficient in vitamin D (25-hydroxyvitamin D 50 to ≤ 125nmol/L).

In table 4.3b, study subjects were stratified into sufficient (25-hydroxyvitamin D equal or more than 75nmol/L and less than 250nmol/L), insufficient (25-hydroxyvitamin D equal or more than 50nmol/L and less than 75nmol/L), and deficient (25-hydroxyvitamin less than 50nmol/L) vitamin D levels as defined by the Endocrine Society and vitamin D experts (Holick et al., 2011). According to this classification, almost half of the sample was considered vitamin D insufficient, while 40.6% of study subjects were vitamin D deficient, and 10.2% were vitamin D sufficient. The percent of study subjects with vitamin D deficiency (25-hydroxyvitamin less than 50nmol/L) in this study sample (40.6%) is comparable to what have been previously reported for healthy normal weight adolescents 11-18 years (55.2%) and obese children and adolescents 7-18 years (42.0%) in the U.S. (Smotkin-Tangorra et al., 2007; Gordon et al., 2004). Furthermore, the percent of study subjects with vitamin D insufficiency (25-hydroxyvitamin D equal or more than 50nmol/L and less than 75nmol/L) in this study sample (49.2%) is similar to what has been reported by the NHANES study 2001-2006 for healthy children 6-11 years (52.0%) (Mansbach et al., 2009).

Table 4.3b Stratification of final sample into sufficient, insufficient and deficient vitamin D status according to the Endocrine Society recommendations (n=197)

		25-hydroxyvitamin D (25-(OH)D; nmol/L) [†] [C]				
	N	Mean 25-(OH)D	Deficiency (%)	Insufficiency (%)	Sufficiency (%)	P-value
All Sample	197	54.6±17.8	40.6	49.2	10.2	
Age at examination, years [C]						***0.001****
Age at examination, years [CAT]						***0.001****
7-12	88	59.3±17.3	29.5	55.7	14.8	
13-18	109	50.8±17.4	49.5	44.0	6.4	
Sex [CAT]						***0.034****
Male	112	56.9±16.7	35.7	53.6	10.7	
Female	85	51.5±18.8	47.1	43.5	9.4	
Ethnicity [CAT]						***0.195***
Caucasian	108	59.3±16.3	30.6	57.4	12.0	
African-American	66	45.0±15.6	59.1	37.9	3.0	
Hispanic	15	55.9±21.4	46.7	33.3	20.0	
Others	8	68.7±15.0	12.5	62.5	25.0	
Insurance status [CAT]						***0.022****
Private	138	56.5±18.0	36.2	51.4	12.3	
Medicaid	59	50.1±16.8	50.8	44.1	5.1	
BMI z-score [C]						***0.486***
BMI Percentile [CAT]						***0.696***
Healthy weight ($< 85^{th}$)	125	54.8±18.9	44.0	43.2	12.8	
Overweight ($\geq 85^{th}$ & $< 95^{th}$)	46	54.9±16.6	32.6	58.7	8.7	
Obese ($\geq 95^{th}$)	26	53.0±15.1	38.5	61.5	-	
Type of Insulin Regimen [CAT]						***0.001****
Insulin Pump	71	58.8±19.2	32.4	53.5	14.1	
MDI, Lantus	59	54.9±16.1	42.4	47.5	10.2	
MDI, NPH	43	52.8±16.3	44.2	48.8	7.0	
Mixed Insulin 2x/day	24	44.6±17.0	54.2	41.7	4.2	
Average Total Daily Insulin Dose, units/kg/day [C]						***0.002****
Season [CAT]						***0.091***
Winter	78	53.5±17.5	44.9	46.2	9.0	
Spring	103	53.7±16.9	41.7	49.5	8.7	
Summer	16	65.5±22.3	12.5	62.5	25.0	

Log Mean Glucose, mg/dl [C]	***0.195***
Log IL-6, pg/ml [C]	***0.031****
Log IL-10, pg/ml [C]	***0.730***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between 25-hydroxyvitamin D (treated as continuous) and select variables. P-value <0.05 is considered significant.

[†] Study sample was stratified into three quartiles according to the Endocrine Society classification for vitamin D status: vitamin D deficiency (25-hydroxyvitamin D < 50 nmol/L), vitamin D insufficiency (25-hydroxyvitamin D 50 to < 75nmol/L), and vitamin D sufficiency (25-hydroxyvitamin D 75 to ≤ 250nmol/L).

Stratification of Final Sample by Inflammatory Cytokines (Tables 4.4a, 4.4b, and 4.4c)

Systemic levels of pro-inflammatory cytokine IL-8 were not detected in this study sample. Mean pro-inflammatory cytokine IL-6 was 1.1±1.2pg/ml for the final study sample (n=197). Pro-inflammatory cytokine IL-6 levels were significantly correlated with age (P=0.038), BMI z-score (P=0.006), anti-inflammatory cytokine IL-10 (P=0.037), and 25-hydroxyvitamin D (P=0.032). Serum IL-6 levels were significantly higher in females as compared to males (P=0.001) and in subjects at risk of vitamin D deficiency as compared to subjects at risk of vitamin D inadequacy or sufficiency (P=0.045). Serum IL-6 levels were not significantly different by race/ethnicity (P=0.244).

Since the primary focus of this study relates to glycemic control which significantly varied by race/ethnicity, univariate associations between serum IL-6 and socio-demographic and disease-related covariates were re-examined separately for Caucasians and African Americans (tables 4.4b and 4.4c). In both Caucasians and African Americans, serum IL-6 levels remained significantly higher in females as compared to males (P=0.007 and P=0.064; respectively). Associations between serum IL-6 and age, anti-inflammatory cytokine IL-10, and 25-hydroxyvitamin D were no longer significant in either subgroup. Interestingly, serum IL-6 retained statistical significance with BMI z-score (P=0.036) in African Americans but not in Caucasians (P=0.239).

Mean anti-inflammatory cytokine IL-10 was 10.5±20.3pg/ml for the final study sample (n=197). Serum IL-10 levels were significantly associated with pro-inflammatory cytokine IL-6 (P=0.037), and HbA1c (P=0.015). Serum IL-10 levels showed a trend towards significance for subjects with diabetes duration ≥ 48 months as compared to

subjects with diabetes duration ≤48 months (P=0.063). In Caucasians, serum IL-10 was no longer associated with HbA1c (P=0.695) or IL-6 (P=0.125). In African Americans, serum IL-10 retained statistical significance with HbA1c (P=0.006) but not with serum IL-6 (P=0.200).

Table 4.4a Univariate associations between inflammatory cytokines and socio-demographic and disease-related characteristics in the final sample (n=197)

		IL-6 (pg/ml) [C]		IL-10 (pg/ml) [C]	
	N	Mean	P-value	Mean	P-value
All Sample	197	1.1±1.2		10.5±20.3	
Age at examination, years [C]			***0.038****		***0.206***
Age at examination, years [CAT]			***0.288***		***0.451***
7-12	88	1.1±1.4		9.5±20.0	
13-18	109	1.1±1.1		11.3±20.6	
Sex [CAT]			***0.001****		***0.917***
Male	112	0.9±1.1		9.8±18.5	
Female	85	1.3±1.4		11.4±22.6	
Ethnicity [CAT]			***0.244***		***0.637***
Caucasian	108	1.0±1.0		10.2±22.1	
African-American	66	1.3±1.7		11.9±20.1	
Hispanic	15	0.7±0.4		6.8±9.0	
Others	8	0.6±0.3		9.5±7.3	
BMI z-score [C]			***0.006****		***0.839***
BMI Percentile [CAT]			***0.068***		***0.541***
Healthy weight (< 85th)	125	1.0±1.2		11.5±23.5	
Overweight (≥ 85th & < 95th)	46	1.2±1.5		9.6±14.6	
Obese (≥ 95th)	26	1.2±1.0		7.3±10.8	
Diabetes duration, months [CAT]			***0.098***		***0.063***
12-23	29	0.9±1.0		7.5±9.5	
24-47	54	1.0±1.0		6.9±14.4	
≥ 48	114	1.2±1.4		13.0±24.2	
Average Total Daily Insulin Dose, units/kg/day [C]			***0.385***		***0.982***
Log Mean Glucose, mg/dl [C]			***0.464***		***0.695***
Log IL-6, pg/ml [C]					***0.037****
Log IL-10, pg/ml [C]			***0.037****		
25-hydroxyvitamin D, nmol/L [C]			***0.032****		***0.730***

25-hydroxyvitamin D, nmol/L [CAT]			***0.045****		***0.781***
At risk of deficiency[£]	18	1.3±1.2		27.7±44.4	
Risk of Inadequacy[£]	62	1.1±1.1		6.2±11.0	
Sufficient-1[£]	97	1.2±1.4		10.0±15.9	
Sufficient-2[£]	20	0.6±0.4		12.1±24.8	
HbA1c, % [C]			***0.253***		***0.015****
HbA1c, % [CAT]			***0.172***		***0.103***
Good[ŧ]	55	0.9±0.97		9.3±21.5	
Intermediate[ŧ]	95	1.1±1.0		10.0±20.8	
Poor[ŧ]	45	1.3±1.8		13.3±18.5	

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between Log-IL-6 (treated as continuous) and select variables. P-value <0.05 is considered significant.

General linear modeling was used to assess univariate associations between Log-IL-10 (treated as continuous) and select variables. P-value <0.05 is considered significant.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

[ŧ]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

Table 4.4b Univariate associations between inflammatory cytokines and socio-demographic and disease-related characteristics in Caucasians (n=108)

		IL-6 (pg/ml) [C]		IL-10 (pg/ml) [C]	
	N	Mean	P-value	Mean	P-value
Caucasian	108	1.0±1.0		10.2±22.1	
Age at examination, years [C]			***0.060***		***0.671***
Age at examination, years [CAT]			***0.244***		***0.767***
7-12	48	0.9±0.9		11.2±25.8	
13-18	60	1.1±1.1		9.4±18.8	
Sex [CAT]			***0.007****		***0.550***
Male	69	0.9±1.0		9.6±20.5	
Female	39	1.2±0.9		11.2±25.1	
BMI z-score [C]			***0.239***		***0.966***
BMI Percentile [CAT]			***0.485***		***0.266***
Healthy weight (< 85th)	72	1.0±0.9		12.4±26.7	
Overweight (≥ 85th & < 95th)	22	1.2±1.4		6.4±5.6	
Obese (≥ 95th)	14	0.9±0.5		4.7±2.7	
Diabetes duration, months [CAT]			***0.223***		***0.334***
12-23	16	0.6±0.4		8.4±10.7	
24-47	30	1.1±0.9		5.4±4.4	
≥ 48	62	1.1±1.1		13.0±28.4	
Average Total Daily Insulin Dose, units/kg/day [C]			***0.579***		***0.465***
Log Mean Glucose, mg/dl [C]			***0.266***		***0.272***
Log IL-6, pg/ml [C]					***0.125***
Log IL-10, pg/ml [C]			***0.125***		
25-hydroxyvitamin D, nmol/L [C]			***0.249***		***0.061***
25-hydroxyvitamin D, nmol/L [CAT]			***0.332***		***0.064***
At risk of deficiency[£]	2	0.7±0.5		77.1±107.1	
Risk of Inadequacy[£]	31	1.1±1.2		4.0±3.0	
Sufficient-1[£]	62	1.1±1.0		10.0±18.2	
Sufficient-2[£]	13	0.5±0.3		17.2±30.7	
HbA1c, % [C]			***0.596***		***0.695***

HbA1c, % [CAT]			***0.361***	***0.712***
Good[ŧ]	40	1.0±1.0	10.9±25.5	
Intermediate[ŧ]	59	1.0±1.1	9.5±22.0	
Poor[ŧ]	9	0.9±0.5	11.1±10.8	

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between Log-IL-6 (treated as continuous) and select variables. P-value <0.05 is considered significant.

General linear modeling was used to assess univariate associations between Log-IL-10 (treated as continuous) and select variables. P-value <0.05 is considered significant.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

[ŧ]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

Table 4.4c Univariate associations between inflammatory cytokines and socio-demographic and disease-related characteristics in African Americans (n=66)

		IL-6 (pg/ml) [C]		IL-10 (pg/ml) [C]	
	N	Mean	P-value	Mean	P-value
African Americans	66	1.3±1.7		11.9±20.1	
Age at examination, years [C]			***0.160***		***0.132***
Age at examination, years [CAT]			***0.486***		***0.279***
7-12	29	1.4±2.1		7.5±9.2	
13-18	37	1.3±1.3		15.5±25.3	
Sex [CAT]			***0.064***		***0.933***
Male	29	1.1±1.5		10.2±16.6	
Female	37	1.6±1.8		13.3±22.6	
BMI z-score [C]			***0.036****		***0.884***
BMI Percentile [CAT]			***0.116***		***0.520***
Healthy weight (< 85th)	40	1.2±1.7		10.9±20.5	
Overweight (≥ 85th & < 95th)	16	1.4±1.8		14.4±22.0	
Obese (≥ 95th)	10	1.7±1.4		11.7±16.7	
Diabetes duration, months [CAT]			***0.579***		***0.139***
12-23	12	1.2±1.3		6.3±8.5	
24-47	16	1.1±1.4		10.9±25.5	
≥ 48	38	1.5±1.9		14.2±20.3	
Average Total Daily Insulin Dose, units/kg/day [C]			***0.546***		***0.300***
Log Mean Glucose, mg/dl [C]			***0.910***		***0.685***
Log IL-6, pg/ml [C]					***0.200***
Log IL-10, pg/ml [C]			***0.200***		
25-hydroxyvitamin D, nmol/L [C]			***0.438***		***0.091***
25-hydroxyvitamin D, nmol/L [CAT]			***0.349***		***0.153***
At risk of deficiency[£]	14	1.5±1.3		23.9±34.0	
Risk of Inadequacy[£]	25	1.1±1.2		8.7±16.7	
Sufficient-1[£]	25	1.5±2.2		9.6±10.8	
Sufficient-2[£]	2	1.1±1.2		1.7±0.82	
HbA1c, % [C]			***0.655***		***0.006****

HbA1c, % [CAT]		***0.588***		***0.085***
Good[ŧ]	10	1.0±1.0	4.4±4.0	
Intermediate[ŧ]	26	1.3±1.1	12.0±21.5	
Poor[ŧ]	28	1.6±2.3	15.2±22.5	

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess univariate associations between Log-IL-6 (treated as continuous) and select variables. P-value <0.05 is considered significant.

General linear modeling was used to assess univariate associations between Log-IL-10 (treated as continuous) and select variables. P-value <0.05 is considered significant.

[£] The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D < 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D 30 to < 50nmol/L), sufficient-1 (25-hydroxyvitamin D 50 to < 75nmol/L), and sufficient-2 (25-hydroxyvitamin D 75nmol/L to ≤ 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since they displayed a different relationship between 25-hydroxyvitamin D and HbA1c.

[ŧ]American Diabetes Association target values for HbA1c stratified by age: HbA1c < 8.0% at age 6 to 12 years and HbA1c < 7.5% at age 13-18 years is classified as good control. HbA1c ≥ 9.5 is classified as poor control for all age groups. HbA1c values between good and poor control are classified as intermediate control.

Table 4.5 Bivariate correlations between continuous variables using Pearson product-moment coefficient (r)

HbA1c	**1**						
Age (years)	**r = 0.11 P = 0.14 n = 195**	**1**					
BMI z-score	**r = 0.10 P = 0.17 n = 195**	**r = 0.15 P = 0.027 n = 197**	**1**				
Insulin Dose (units/kg/day)	**r = 0.26 P = 0.002 n = 195**	**r = 0.24 P = 0.0007 n = 197**	**r = 0.04 P = 0.57 n = 197**	**1**			
25-hydroxyvitamin D (nmol/L)	**r = -0.15 P = 0.037 n = 195**	**r = -0.24 P = 0.0008 n = 197**	**r = -0.05 P = 0.48 n = 197**	**r = -0.22 P = 0.002 n = 197**	**1**		
Log Glucose level	**r = 0.20 P = 0.005 n = 190**	**r = -0.08 P = 0.25 n = 192**	**r = 0.002 P = 0.97 n = 192**	**r = 0.11 P = 0.13 n = 192**	**r = -0.09 P = 0.20 n = 192**	**1**	
Log IL-6	**r = 0.06 P = 0.37 n = 191**	**r = 0.14 P = 0.054 n = 193**	**r = 0.19 P = 0.007 n = 193**	**r = 0.05 P = 0.46 n = 193**	**r = -0.14 P = 0.053 n = 193**	**r = -0.06 P = 0.38 n = 192**	**1**
Log IL-10	**r = 0.18 P = 0.012 n = 189**	**r = 0.09 P = 0.19 n = 191**	**r = -0.01 P = 0.85 n = 191**	**r = -0.001 P = 0.99 n = 191**	**r = 0.02 P = 0.79 n = 191**	**r = -0.02 P = 0.8 n = 190**	**r = 0.15 P = 0.034 n = 191**

Aim One (Tables 4.6a, 4.6b and 4.6c)

The primary aim of this study was to quantify the relationship between 25-hydroxyvitamin D and HbA1c excluding inflammatory cytokines (IL-6, IL-8, and IL-10). It was hypothesized that 25-hydroxyvitamin D is inversely related to HbA1c (table 4.6a). General linear modeling (GLM) was used to examine this relationship. Assumptions required for inferences from GLM analyses were met. The histogram of estimated residuals was closer to a bell-shape and the normal probability plots (P-P plot and Q-Q plot) of estimated residuals approximated a straight line suggesting no significant departures from normality. Also the scatter plot of residuals versus fitted values showed constant variance of residuals across the range of fitted values. Statistical significance was set at $P< 0.05$.

In the unadjusted model; 25-hydroxyvitamin D was inversely correlated to HbA1c, and almost reached statistical significance (β=-0.01; P=0.055). In model 2; after adjusting for socio-demographic covariates, 25-hydroxyvitamin D remained inversely correlated to HbA1c but was not statistically significant (β=-0.005; P=0.377). Also, in model 3, after adjusting for socio-demographic and disease-related covariates, the relationship between 25-hydroxyvitamin D and HbA1c was not significant (β=0.008; P=0.108).

Based on univariate analyses, both HbA1c and 25-hydroxyvitamin D were significantly different by race/ethnicity (tables 4.2b and 4.2c). Furthermore, a previous study examining the relationship between 25-hydroxyvitamin D and HbA1c in obese children and adolescent across three different races, showed an inverse and significant association in Caucasians, a trend towards significance in Hispanics, and a non-

significant association in African Americans (Alemzadeh et al., 2008). For this study sample, it may be plausible that the significant relationship between 25-hydroxyvitamin D and HbA1c observed in the unadjusted model (β=-0.01; P=0.055) is modified by race/ethnicity. Therefore, the relationship between 25-hydroxyvitamin D and HbA1c was re-examined in Caucasians and African Americans separately (tables 4.6b and 4.6c; Figure 4.6). Similar to the final study sample, the adjusted relationship between 25-hydroxyvitamin D and HbA1c was not significant for both Caucasians and African Americans. Figure 4.6 showed no significant difference in the slope of 25-hydroxyvitamin D by race (β=-0.003; P=0.775).

In the multivariate model for final study sample (n = 197; table 4.6a), HbA1c was significantly associated with ethnicity (β=0.24; P=0.023), insurance status (β =0.40; P=0.031), diabetes duration (β =0.38; P=0.001), type of insulin regimen (β=0.28; P=0.001), average total daily insulin dose (β=0.68; P=0.014), FBGM (β=-0.58; P=0.000), frequency of nurse practitioner clinic visit over the past year (β=-0.34; P=0.042), and blood glucose level (β=0.30; P=0.028). Furthermore, predictors of glycemic control as measured by HbA1c were different for Caucasians and African Americans (tables 4.6b and table 4.6c). In adjusted model for Caucasians, HbA1c was significantly associated with insurance status (β=0.55; P=0.047), BMI z-score (β=0.26; P=0.042), average total daily insulin dose (β=0.76; P=0.014), and FBGM (β=-0.65; P<0.000). Conversely, in adjusted model for African Americans, HbA1c was significantly associated with diabetes duration (β=0.58; P=0.010), type of insulin regimen (β=0.38; P=0.013), FBGM (β=-0.53; P=0.055), and frequency of nurse practitioner clinic visit over the past year (β=-0.77; P=0.030).

Table 4.6a HbA1c regressed on 25-hydroxyvitamin D in the final sample (n = 197)

	Model 1 **(F=3.68, P=0.055) Coefficient, *P***	Model 2 **(F=5.10, P=0.000) Coefficient, *P***	Model 3 **(F=9.71, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.05, ***0.129***	-0.02, ***0.632***
Sex [CAT]		0.33, ***0.078***	-0.01, ***0.957***
Ethnicity [CAT]		0.38, ***0.001****	0.24, ***0.023****
Insurance status [CAT]		0.70, ***0.001****	0.40, ***0.031****
BMI z-score [C]		0.08, ***0.490***	0.02, ***0.831***
Season [CAT]		-0.09, ***0.571***	-0.15, ***0.281***
Diabetes duration, months [CAT]			0.38, ***0.001****
Type of Insulin Regimen [CAT]			0.28, ***0.001****
Average Total Daily Insulin Dose, units/kg/day [C]			0.68, ***0.014****
Frequency of Blood Glucose Monitoring per Day [CAT]			-0.58, ***0.000****
Frequency of CRNP Clinic Visit over the past Year [CAT]			-0.34, ***0.042****
25-hydroxyvitamin D, nmol/L [C]	-0.01, ***0.055****	-0.005, ***0.377***	0.008, ***0.108***
Log Mean Glucose, mg/dl [C]			0.30, ***0.028****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Table 4.6b HbA1c regressed on 25-hydroxyvitamin D in Caucasians (n = 108)

	Model 1 **(F=0.00, P=0.966) Coefficient, *P***	Model 2 **(F=2.87, P=0.009) Coefficient, *P***	Model 3 **(F=4.13, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.04, ***0.264***	-0.01, ***0.818***
Sex [CAT]		0.40, ***0.056***	0.22, ***0.263***
Insurance status [CAT]		0.60, ***0.035****	0.55, ***0.047****
BMI z-score [C]		0.26, ***0.056***	0.26, ***0.042****
Season [CAT]		-0.24, ***0.188***	-0.25, ***0.147***
Diabetes duration, months [CAT]			0.11, ***0.443***
Type of Insulin Regimen [CAT]			-0.09, ***0.500***
Average Total Daily Insulin Dose, units/kg/day [C]			0.76, ***0.014****
Frequency of Blood Glucose Monitoring per Day [CAT]			-0.65, ***0.000*** **
Frequency of CRNP Clinic Visit over the past Year [CAT]			-0.13, ***0.494***
25-hydroxyvitamin D, nmol/L [C]	-0.0002, ***0.966***	0.03, ***0.682***	0.009, ***0.109***
Log Mean Glucose, mg/dl [C]			0.27, ***0.090***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

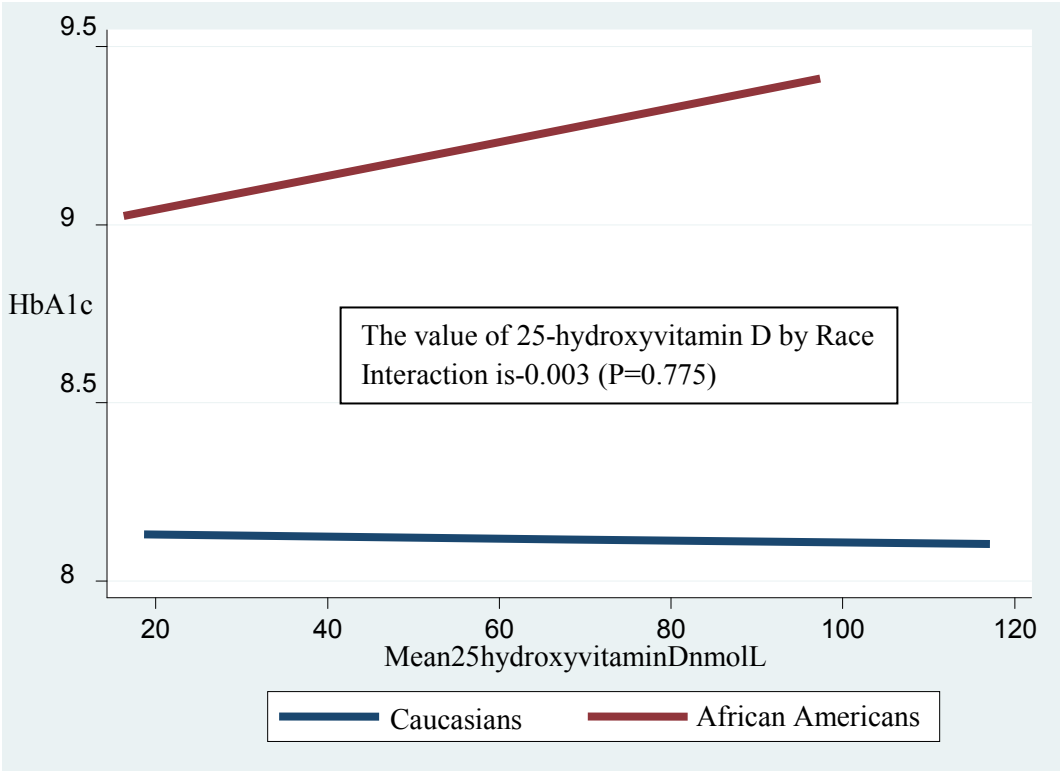
Table 4.6c HbA1c regressed on 25-hydroxyvitamin D in African Americans (n = 66)

	Model 1 **(F=0.31, P=0.576) Coefficient, *P***	Model 2 **(F=0.47, P=0.828) Coefficient, *P***	Model 3 **(F=3.33, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.08, ***0.290***	-0.05, ***0.474***
Sex [CAT]		0.09, ***0.818***	-0.31, ***0.363***
Insurance status [CAT]		0.33, ***0.393***	0.36, ***0.263***
BMI z-score [C]		-0.12, ***0.612***	-0.23, ***0.259***
Season [CAT]		0.25, ***0.412***	-0.21, ***0.464***
Diabetes duration, months [CAT]			0.58, ***0.010****
Type of Insulin Regimen [CAT]			0.38, ***0.013****
Average Total Daily Insulin Dose, units/kg/day [C]			0.97, ***0.105***
Frequency of Blood Glucose Monitoring per Day [CAT]			-0.53, ***0.055****
Frequency of CRNP Clinic Visit over the past Year [CAT]			-0.77, ***0.030***
25-hydroxyvitamin D, nmol/L [C]	0.007, ***0.576***	0.01, ***0.411***	0.012, ***0.300***
Log Mean Glucose, mg/dl [C]			0.45, ***0.093***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.6 Examining the relationship between 25-hydroxyvitamin D and HbA1c by Race



Although the relationship between 25-hydroxyvitamin D and HbA1c was not significant based on previous analyses, the following sections re-examine this relationship across different vitamin D levels. The study sample was stratified into four quartiles according to the IOM classification for vitamin D status: at risk of deficiency (25-hydroxyvitamin D less than 30 nmol/L), risk of inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L), sufficient-1 (25-hydroxyvitamin D equal or more than 50 and less than 75nmol/L), and sufficient-2 (25-hydroxyvitamin D equal or more than 75nmol/L and less or equal to 125nmol/L). Patients classified sufficient in vitamin D were divided into two groups since the relationship between 25-hydroxyvitamin D and HbA1c changed at 25-hydroxyvitamin D level of 75nmol/L. These findings may be interesting given the definition of vitamin D sufficiency (25-hydroxyvitamin D equal ore more than 75 and equal or less than 250nmol/L) by the Endocrine Society and vitamin D experts. However, experimental research is needed to confirm these results.

Table 4.7a shows the best model examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D deficiency (25-hydroxyvitamin D less than 30nmol/L). Only 18 study subjects had 25-hydroxyvitamin D levels less than 30nmol/L. After close examination of the racial/ethnic distribution; two out 18 were Caucasian, two out of 18 were Hispanic, and the remaining majority were African American (14 out of 18). Furthermore, from figure 4.7a which is a scatter plot that graphically displays the relationship between 25-hydroxyvitamin D and HbA1c, the majority of study subjects had HbA1c levels higher than 8.0% i.e. had either intermediate or poor glycemic control. In this subgroup, the relationship between 25-hydroxyvitamin

D and HbA1c was significant (P=0.035) suggesting that African Americans are more likely to have poor glycemic control and low levels of 25-hydroxyvitamin D concurrently. However, the strength and directionality of this relationship (β=0.17) should be interpreted with caution since the sample size is small (n=18) and therefore lacks the statistical power to detect meaningful associations.

Table 4.7a HbA1c regressed on 25-hydroxyvitamin D in individuals at risk of vitamin D deficiency (25-hydroxyvitamin D less than 30nmol/L) (n =18)

	Best fit Model **(F=4.81, P=0.000)** **Coefficient, *P***
Insurance status [CAT]	1.18, ***0.038****
Diabetes duration, months [CAT]	1.02, ***0.020****
Type of Insulin Regimen [CAT]	0.45, ***0.056***
Average Total Daily Insulin Dose, units/kg/day [C]	2.00, ***0.028****
25-hydroxyvitamin D, nmol/L[C]	0.17, ***0.035****
Log Mean Glucose, mg/dl [C]	1.10, ***0.030****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7a Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D deficiency (25-hydroxyvitamin D less than 30nmol/L)

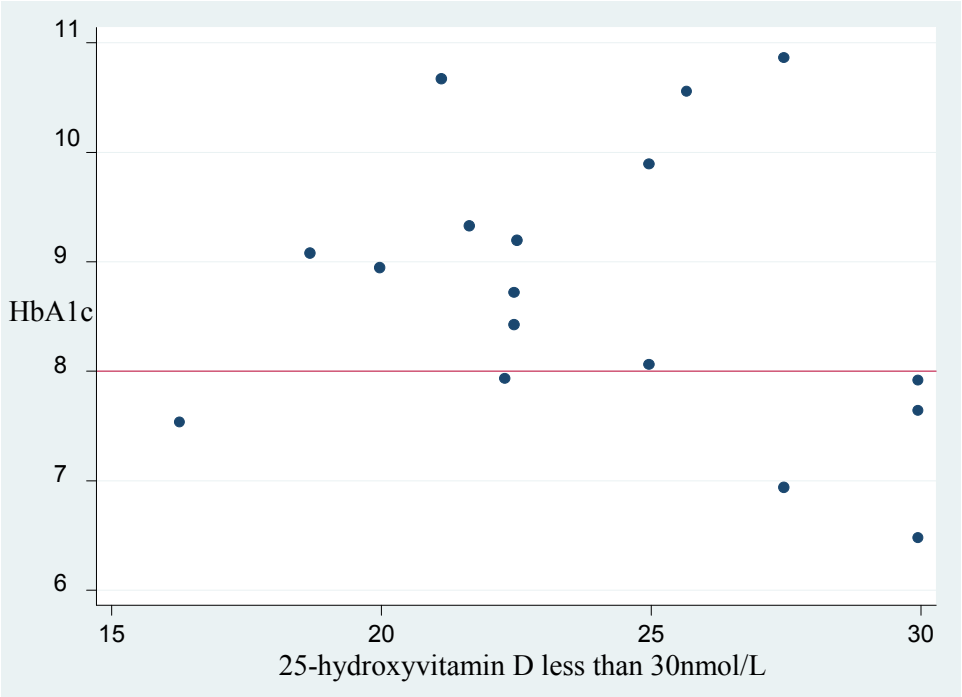


Table 4.7b shows the best model examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L). A total of 62 study subjects were at risk of vitamin D inadequacy. In this subgroup, 25-hydroxyvitamin D was not associated with HbA1c (β=-0.008, P=0.772). However, after examining the relationship between 25-hydroxyvitamin D and HbA1c by race the relationship was found to be significant for Caucasians (n=31; β=-0.07; P=0.023) but remained not significant for African Americans (n=25; β=0.06; P=0.276) (tables 4.7b1 and 4.7b2). Although there is no statistical power at such small sample size to make a definitive conclusion about observed relationships; however, if those relationships do exist it may be reasonable to conclude that Caucasians and African Americans may have different physiologic mechanisms that regulate the relationship between 25-hydroxyvitamin D and HbA1c.

Table 4.7b HbA1c regressed on 25-hydroxyvitamin D in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L) (n =62)

	Best fit Model **(F=5.22, P=0.000)** **Coefficient, *P***
Ethnicity [CAT]	0.74, ***0.001****
Average Total Daily Insulin Dose, units/kg/day [C]	1.13, ***0.018****
Frequency of Blood Glucose Monitoring per Day [CAT]	-0.54, ***0.025****
25-hydroxyvitamin D, nmol/L [C]	-0.008, ***0.772***
Log Mean Glucose, mg/dl [C]	0.49, ***0.063***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7b Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L)

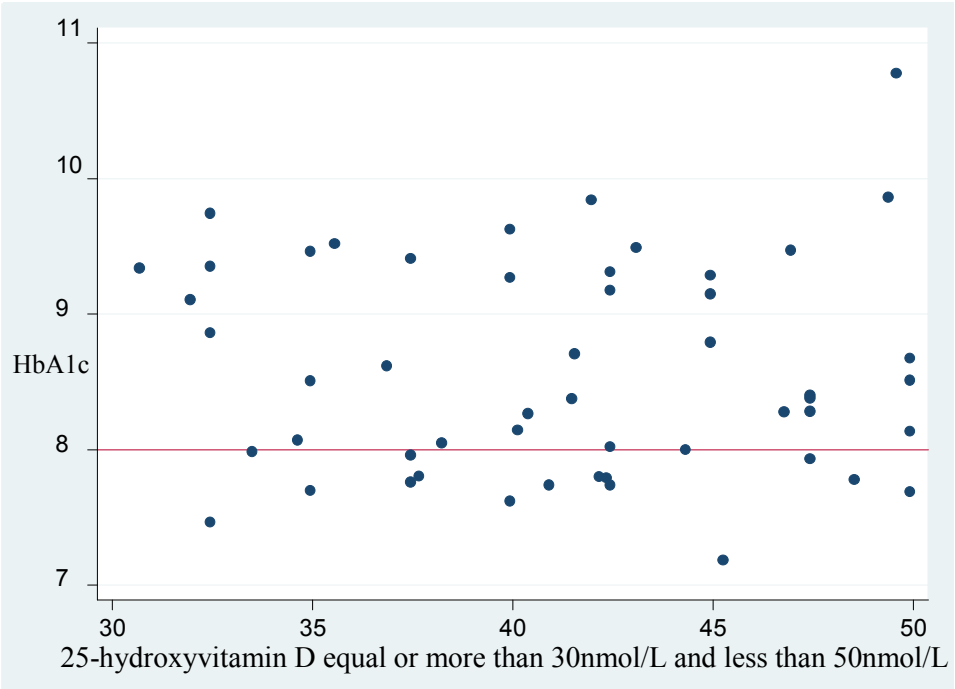


Table 4.7b1 HbA1c regressed on 25-hydroxyvitamin D in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L) in Caucasians (n =31)

	Best fit Model **(F=6.18, P=0.000)** **Coefficient, *P***
Average Total Daily Insulin Dose, units/kg/day [C]	1.38, ***0.011****
25-hydroxyvitamin D, nmol/L[C]	-0.07, ***0.023****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7b1 Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L) in Caucasians

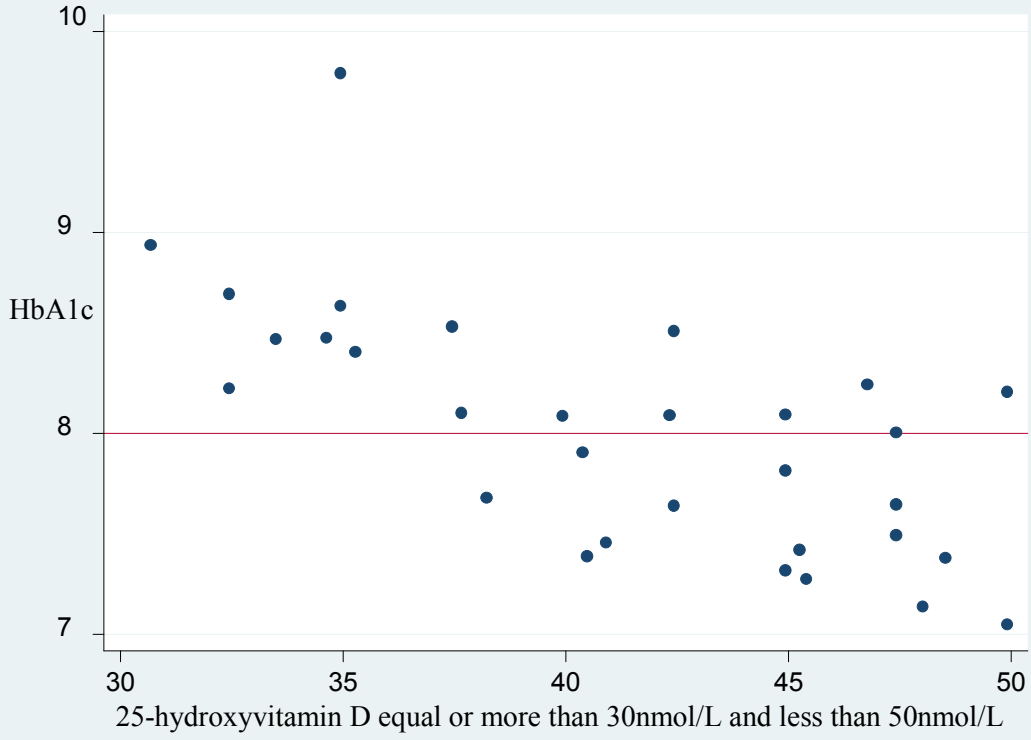


Table 4.7b2 HbA1c regressed on 25-hydroxyvitamin D in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L) in African Americans (n =25)

	Best fit Model **(F=2.18, P=0.113)** **Coefficient, *P***
25-hydroxyvitamin D, nmol/L[C]	0.06, ***0.276***
Log Mean Glucose, mg/dl [C]	0.92, ***0.050****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7b2 Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals at risk of vitamin D inadequacy (25-hydroxyvitamin D equal or more than 30nmol/L and less than 50nmol/L) in African Americans

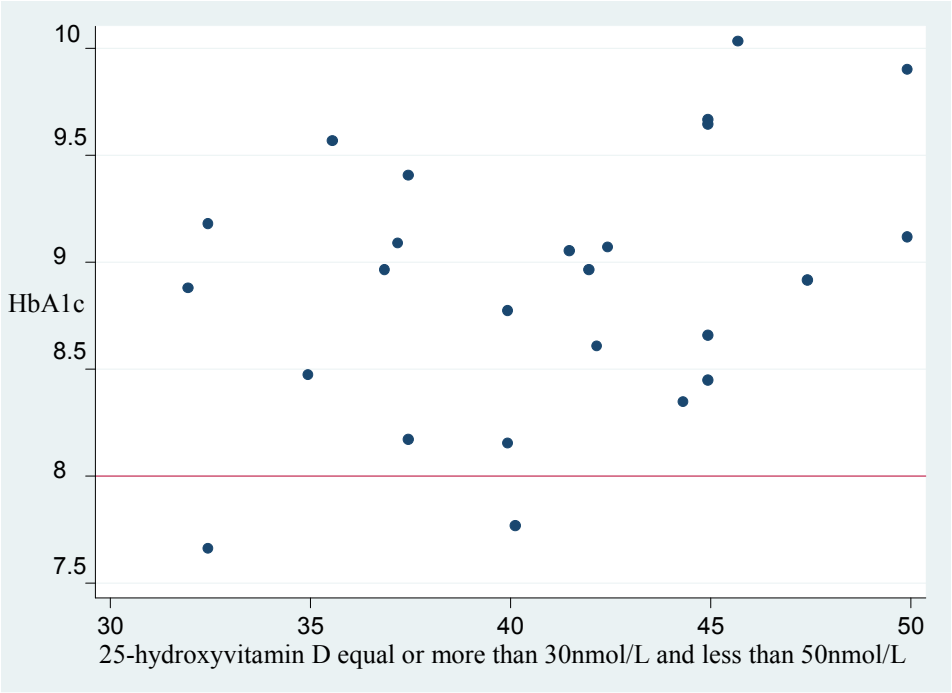


Table 4.7c reports the best fit model examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal or more than 50nmol/L and less than 75nmol/L). A total of 97 study subjects were vitamin D sufficient. In this subgroup 25-hydroxyvitamin D was inversely associated with HbA1c (β=-0.04, P=0.014). When looking at this relationship by ethnicity, 25-hydroxyvitamin D remained statistically significant for African Americans (n=25; β=-0.08; P=0.044) but lost significance for Caucasians (n=62; β=-0.0009; P=0.961) (tables 4.7c1 and 4.7c2). Similar to prior analyses, there is no statistical power at such small sample size to make a definitive conclusion about observed relationships. However, it may be hypothesized that African Americans require higher levels of vitamin D as compared to Caucasians to observe an effect on glycemic control. Interventions studies are needed to test this hypothesis.

Table 4.7c HbA1c regressed on 25-hydroxyvitamin D in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L) (n =97)

	Best fit Model **(F=19.53, P=0.000)** **Coefficient, *P***
Diabetes duration, months [CAT]	0.31, ***0.037****
Type of Insulin Regimen [CAT]	0.43, ***0.000****
Frequency of Blood Glucose Monitoring per Day [CAT]	-0.73, ***0.000****
25-hydroxyvitamin D, nmol/L[C]	-0.04, ***0.014****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7c Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L)

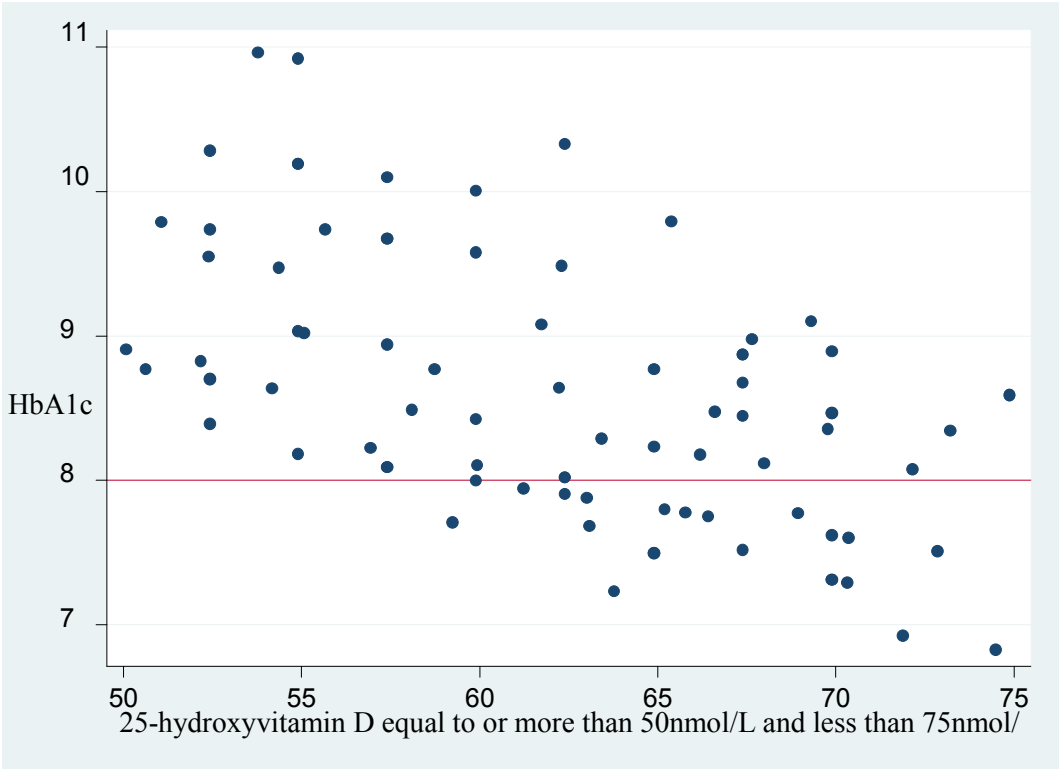


Table 4.7c1 HbA1c regressed on 25-hydroxyvitamin D in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L) in Caucasians (n =62)

	Best fit Model **(F=8.32, P=0.000)** **Coefficient, *P***
Type of Insulin Regimen [CAT]	0.34, ***0.022****
Frequency of Blood Glucose Monitoring per Day [CAT]	-0.82, ***0.000****
25-hydroxyvitamin D, nmol/L[C]	-0.0009, ***0.961****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7c1 Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L) in Caucasians

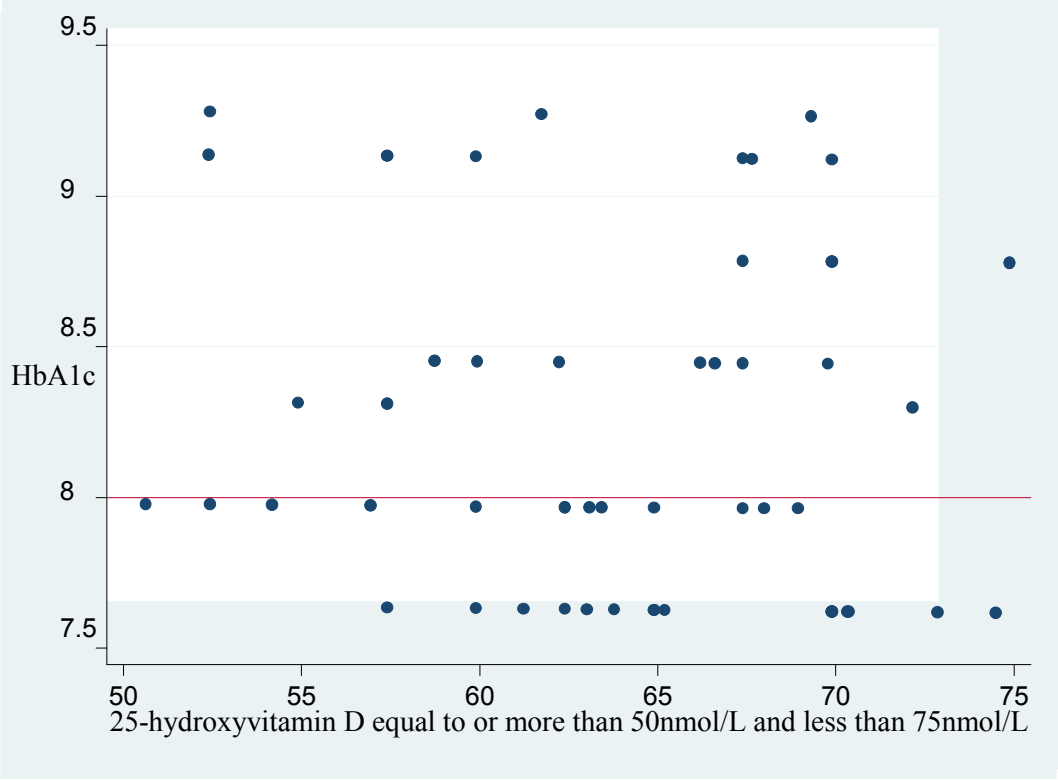


Table 4.7c2 HbA1c regressed on 25-hydroxyvitamin D in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L) in African Americans (n=25)

	Best fit Model **(F=6.37, P=0.000)** **Coefficient, *P***
Diabetes duration, months [CAT]	0.71, ***0.023****
Type of Insulin Regimen [CAT]	0.53, ***0.003****
25-hydroxyvitamin D, nmol/L [C]	-0.08, ***0.044****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7c2 Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-1 (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 75nmol/L) in African Americans

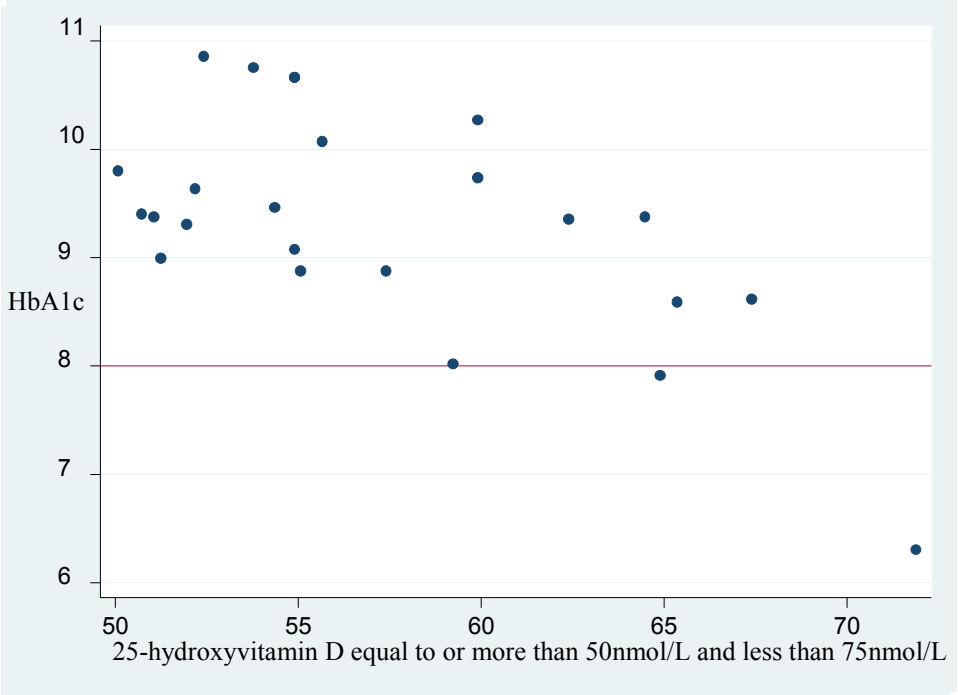


Table 4.7d shows the best model examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-2 (25-hydroxyvitamin D greater than 75nmol/L). A total of 20 study subjects had 25-hydroxyvitamin D levels greater than 75nmol/L. 13 were Caucasians, 2 were African Americans, 3 were Hispanic, and 2 were Others. In this subgroup 25-hydroxyvitamin D was not significantly associated with HbA1c (β =-0.02, P=0.424) although the β coefficient was negative. Intervention studies are needed to confirm these results.

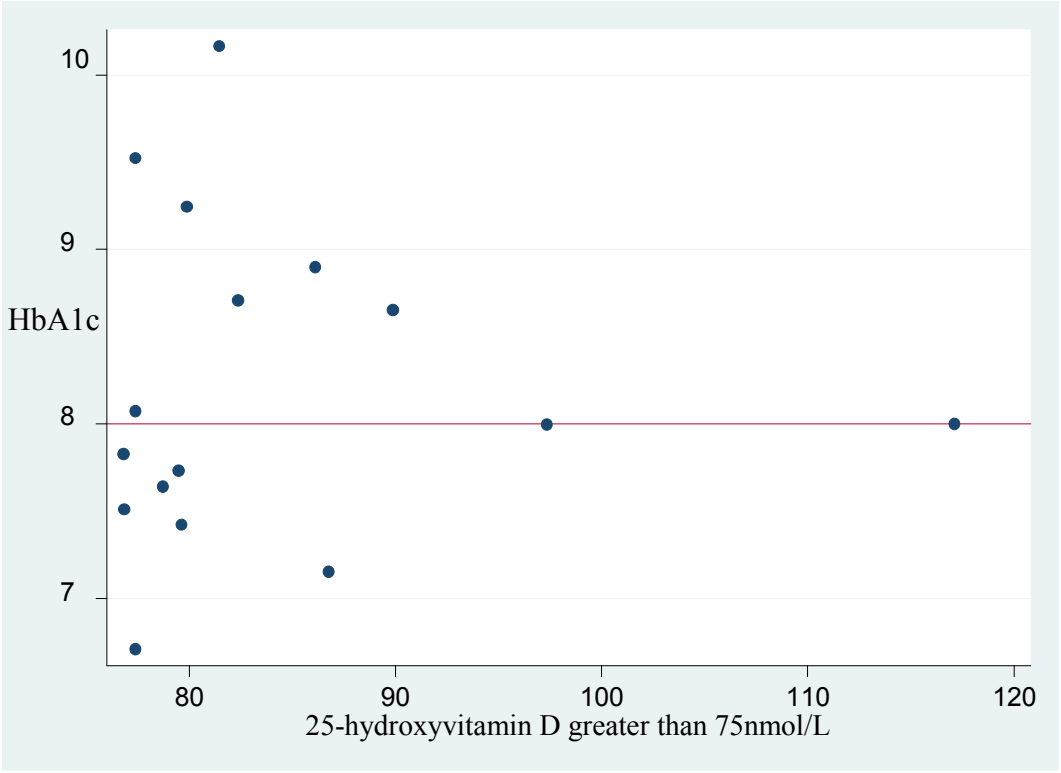
Table 4.7d HbA1c regressed on 25-hydroxyvitamin D in individuals who are vitamin D sufficient-2 (25-hydroxyvitamin D greater than 75nmol/L) (n =20)

	Best fit Model **(F=2.73, P=0.028)** **Coefficient, *P***
Type of Insulin Regimen [CAT]	0.66, ***0.028****
Frequency of CRNP Clinic Visit over the past Year [CAT]	-1.87, ***0.035****
25-hydroxyvitamin D, nmol/L[C]	-0.02, ***0.424***
Log Mean Glucose, mg/dl [C]	1.28, ***0.036****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between HbA1c (treated as continuous) and 25-hydroxyvitamin D (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Figure 4.7d Scatter plot examining the relationship between 25-hydroxyvitamin D and HbA1c in individuals who are vitamin D sufficient-2 (25-hydroxyvitamin D greater than 75nmol/L)



Aim Two (Table 4.8)

The secondary aim of this study was to quantify the relationship between 25-hydroxyvitamin D and IL-6 while excluding HbA1c and other inflammatory markers (IL-10). It was hypothesized that 25-hydroxyvitamin D is inversely correlated to IL-6 (table 4.8). GLM was used to examine this relationship. Assumptions required for inferences from GLM analyses were met. The histogram of estimated residuals was closer to a bell-shape and the normal probability plots (P-P plot and Q-Q plot) of estimated residuals approximated a straight line suggesting no significant departures from normality. Also the scatter plot of residuals versus fitted values showed constant variance of residuals across the range of fitted values. Statistical significance was set at $P< 0.05$.

In the unadjusted model; 25-hydroxyvitamin D was inversely correlated to IL-6 (β=-0.007; P=0.030). In model 2; after adjusting for socio-demographic covariates, 25-hydroxyvitamin D remained inversely correlated to IL-6 but was no longer statistically significant (β=-0.005; P=0.179). In model 3, the relationship between 25-hydroxyvitamin D and IL-6 was not significant after adjusting for socio-demographic and disease-related covariates (β=-0.005; P=0.175).

Table 4.8 Log IL-6 regressed on 25-hydroxyvitamin D in the final sample (n = 197)

	Model 1 **(F=4.73, P=0.030) Coefficient, *P***	Model 2 **(F=3.97, P=0.000) Coefficient, *P***	Model 3 **(F=3.51, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.02, ***0.311***	0.01, ***0.666***
Sex [CAT]		-0.34, ***0.003*****	-0.36, ***0.002*****
Ethnicity [CAT]		-0.10, ***0.185***	-0.11, ***0.153***
Insurance status [CAT]		-0.002, ***0.988***	0.03; ***0.815***
BMI z-score [C]		0.16, ***0.030****	0.14, ***0.053****
Season [CAT]		-0.14, ***0.140***	-0.17, ***0.076***
Diabetes duration, months [CAT]			0.11, ***0.164***
25-hydroxyvitamin D, nmol/L [C]	-0.007, ***0.030***	-0.005, ***0.179***	-0.005, ***0.175***
Log Mean Glucose, mg/dl [C]			-0.11, ***0.232***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between 25-hydroxyvitamin D (treated as continuous) and IL-6 (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Aim Two (Table 4.9)

The secondary aim of this study was to quantify the relationship between 25-hydroxyvitamin D and IL-10 while excluding HbA1c and other inflammatory markers (IL-6). It was hypothesized that 25-hydroxyvitamin D is directly correlated to IL-10 (table 4.9). GLM was use to examine this relationship. Assumptions required for inferences from GLM analyses were met. The histogram of estimated residuals was closer to a bell-shape and the normal probability plots (P-P plot and Q-Q plot) of estimated residuals approximated a straight line suggesting no significant departures from normality. Also the scatter plot of residuals versus fitted values showed constant variance of residuals across the range of fitted values. Statistical significance was set at $P< 0.05$.

In the unadjusted model; 25-hydroxyvitamin D was inversely correlated to IL-10, although the relationship was not significant (β=-0.002; P=0.729). In model 2; after adjusting for socio-demographic covariates, the relationship between 25-hydroxyvitamin D remained not significant (β=0.004; P=0.426). In model 3, the relationship between 25-hydroxyvitamin D and IL-10 was also not significant after adjusting for socio-demographic and disease-related covariates (β=0.004; P=0.356).

Table 4.9 Log IL-10 regressed on 25-hydroxyvitamin D in the final sample (n = 197)

	Model 1 **(F=0.12, P=0.729) Coefficient, *P***	Model 2 **(F=0.56, P=0.787) Coefficient, *P***	Model 3 **(F=0.91, P=0.505) Coefficient, *P***
Age at examination, years [C]		0.04, ***0.185***	0.02, ***0.474***
Sex [CAT]		-0.03, ***0.852***	-0.06; ***0.691***
Ethnicity [CAT]		0.09, ***0.382***	0.08, ***0.410***
Insurance status [CAT]		-0.02, ***0.897***	-0.003, ***0.986***
BMI z-score [C]		-0.04, ***0.669***	-0.07, ***0.470***
Season [CAT]		-0.15, ***0.255***	-0.18, ***0.153***
Diabetes duration, months [CAT]			0.20, ***0.062***
25-hydroxyvitamin D, nmol/L [C]	-0.002, ***0.729***	0.004, ***0.426***	0.004, ***0.356***

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between 25-hydroxyvitamin D (treated as continuous) and IL-10 (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Aim Three (Table 4.10)

The secondary aim of this study was to quantify the relationship between HbA1c and IL-6 while excluding 25-hydroxyvitamin D and other inflammatory markers (IL-10). It was hypothesized that IL-6 is directly correlated to HbA1c (table 4.10). GLM was used to examine this relationship. Assumptions required for inferences from GLM analyses were met. The histogram of estimated residuals was closer to a bell-shape and the normal probability plots (P-P plot and Q-Q plot) of estimated residuals approximated a straight line suggesting no significant departures from normality. Also the scatter plot of residuals versus fitted values showed constant variance of residuals across the range of fitted values. Statistical significance was set at $P< 0.05$.

In the unadjusted model; IL-6 was directly correlated to HbA1c, although the relationship was not significant ($\beta=0.14$; $P=0.252$). In model 2; after adjusting for socio-demographic covariates, IL-6 remained directly correlated to HbA1c and the relationship was not significant ($\beta=0.17$; $P=0.145$). In model 3, the relationship between IL-6 and HbA1c remained not significant after adjusting for socio-demographic and disease-related covariates ($\beta=0.09$; $P=0.418$).

Table 4.10 HbA1c regressed on Log IL-6 in the final sample (n = 197)

	Model 1 **(F=1.31, P=0.252) Coefficient, *P***	Model 2 **(F=6.19, P=0.000) Coefficient, *P***	Model 3 **(F=10.13, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.06, ***0.080***	-0.02, ***0.568***
Sex [CAT]		0.37, ***0.052***	0.08, ***0.630***
Ethnicity [CAT]		0.40, ***0.001****	0.23, ***0.030****
Insurance status [CAT]		0.73, ***0.000****	0.39, ***0.036****
BMI z-score [C]		0.05, ***0.678***	0.02, ***0.877***
Diabetes duration, months [CAT]			0.35, ***0.003****
Type of Insulin Regimen [CAT]			0.26, ***0.002****
Average Total Daily Insulin Dose, units/kg/day [C]			0.59, ***0.030****
Frequency of Blood Glucose Monitoring per Day [CAT]			-0.54, ***0.000****
Frequency of CRNP Clinic Visit over the past Year [CAT]			-0.30, ***0.066***
Log IL-6[C]	0.14, ***0.252***	0.17, ***0.145***	0.09, ***0.418***
Log Mean Glucose, mg/dl [C]			0.31, ***0.025****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between IL-6 (treated as continuous) and HbA1c (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

Aim Three (Table 4.11)

The secondary aim of this study was to quantify the relationship between HbA1c and IL-10 while excluding 25-hydroxyvitamin D and other inflammatory markers (IL-6). It was hypothesized that IL-10 is inversely correlated to HbA1c (table 4.11). GLM was used to examine this relationship. Assumptions required for inferences from GLM analyses were met. The histogram of estimated residuals was closer to a bell-shape and the normal probability plots (P-P plot and Q-Q plot) of estimated residuals approximated a straight line suggesting no significant departures from normality. Also the scatter plot of residuals versus fitted values showed constant variance of residuals across the range of fitted values. Statistical significance was set at $P< 0.05$.

In the unadjusted model; HbA1c was directly correlated to IL-10 ($\beta=0.24$; $P=0.015$). In model 2; after adjusting for socio-demographic covariates, HbA1c remained directly correlated to IL-10 ($\beta=0.22$; $P=0.018$). In model 3, the relationship between HbA1c and IL-10 remained significant after adjusting for socio-demographic and disease-related covariates ($\beta=0.21$; $P=0.008$).

Table 4.11 HbA1c regressed on Log IL-10 in the final sample (n = 197)

	Model 1 **(F=5.96, P=0.015) Coefficient, *P***	Model 2 **(F=6.88, P=0.000) Coefficient, *P***	Model 3 **(F=11.06, P=0.000) Coefficient, *P***
Age at examination, years [C]		0.06, ***0.087***	-0.02, ***0.449***
Sex [CAT]		0.31, ***0.091***	0.05, ***0.764***
Ethnicity [CAT]		0.37, ***0.002****	0.21, ***0.043****
Insurance status [CAT]		0.75, ***0.000****	0.39, ***0.029****
BMI z-score [C]		0.09, ***0.469***	0.04, ***0.692***
Diabetes duration, months [CAT]			0.32, ***0.007****
Type of Insulin Regimen [CAT]			0.24, ***0.004****
Average Total Daily Insulin Dose, units/kg/day [C]			0.62, ***0.020****
Frequency of Blood Glucose Monitoring per Day [CAT]			-0.58, ***0.000****
Frequency of CRNP Clinic Visit over the past Year [CAT]			-0.33, ***0.039****
Log IL-10[C]	0.24, ***0.015****	0.22, ***0.018****	0.21, ***0.008****
Log Mean Glucose, mg/dl [C]			0.31, ***0.022****

[C], continuous variables; [CAT], categorical variables; *P <0.05, ** P < 0.01

General linear modeling was used to assess the relationship between IL-10 (treated as continuous) and HbA1c (treated as continuous) while adjusting for select covariates. P-value <0.05 is considered significant.

CHAPTER FIVE: DISCUSSION AND CONCLUSIONS

The purpose of this study was to examine the relationship between 25-hydroxyvitamin D (the functional indicator of vitamin D status) and HbA1c (the standardized index of glycemic control); and to determine whether inflammatory cytokines IL-6, IL-8, and IL-10 mediate this relationship in metabolically stable children and adolescents with T1DM. A cross-sectional design was used to examine these relationships in a convenience sample of 197 children and adolescents with T1DM 7-18 years recruited from the Diabetes Center for Children at the Children's Hospital of Philadelphia. Study subjects were recruited between January and June, 2011. In this study sample of children and adolescents with T1DM, neither 25-hydroxyvitamin D nor pro-inflammatory cytokine IL-6 were significantly associated with HbA1c. Conversely, anti-inflammatory cytokine IL-10 was positively associated with HbA1c (β=0.12, P=0.039). Furthermore, serum levels of pro-inflammatory cytokine IL-8 were not detected in this sample.

Key Findings

The Relationship between 25-hydroxyvitamin D and HbA1c in Metabolically Stable Children and Adolescents with T1DM

The primary aim of this study was to examine whether there is a significantly inverse relationship between 25-hydroxyvitamin D and HbA1c in metabolically stable children and adolescents with T1DM. This relationship has been previously reported in obese/Caucasian children and adolescents (Alemzadeh et al., 2008). Also, 25-hydroxyvitamin D has been linked to fasting glucose levels and proxy measures of insulin sensitivity/resistance in healthy children and adolescents (Delvin et al., 2010;

Johnson et al., 2010). Furthermore, vitamin D deficiency has been associated with the increased risk of developing T1DM where the immune modulatory role of 25-hydroxyvitamin D has been suggested as the underlying mechanism explaining this relationship (Levy-Marchal et al., 1995; Mohr et al., 2008; Gysemans et al., 2005; Mathieu et al., 1992; Giulietti et al., 2004; Gregori et al., 2002; Mathieu et al., 1994; Simpson et al., 2011). In addition, vitamin D deficiency has been considered one of many environmental risk factors involved in the pathogenesis of T2DM through its effect on insulin secretion and insulin sensitivity (Scragg et al., 2004; Chiu et al., 2004; Knekt et al., 2008; Liu et al., 2010).

To my knowledge, this is the first study to examine the relationship between 25-hydroxyvitamin D and HbA1c in metabolically stable children and adolescents with T1DM after the first year of diagnosis. Only one previous study examined the relationship between 25-hydroxyvitamin D and HbA1c in youth recently diagnosed with T1DM and youth with established T1DM (Svoren et al., 2009). In bivariate analyses, vitamin D deficiency (25-hydroxyvitamin D less than 50 nmol/L) was associated with lower levels of HbA1c (P=0.05), although the relationship was no longer significant after adjusting for age, sex, ethnicity, season, BMI z-score, and diabetes duration (Svoren et al., 2009). Similarly, in this study sample, there was a trend of decreasing levels of HbA1c as 25-hydroxyvitamin D levels improved from levels at risk of deficiency (25-hydroxyvitamin D less than 30nmol/L) towards sufficient states (25-hydroxyvitamin D equal to or more than 50nmol/L and less than 125nmol/L). The unadjusted relationship between HbA1c and 25-hydroxyvitamin D almost reached statistical significance (β=-

0.01, P=0.055), but was no longer significant after adjusting for socio-demographic and disease-related covariates (β=0.008, P=0.108).

Several reasons may explain the non-significant association observed between 25-hydroxyvitamin D and HbA1c in this study sample. I excluded patients with HbA1c levels ≥12.0% and therefore my study sample was not reflective of the full spectrum of patients with poor glycemic control in T1DM population (HbA1c ≥ 9.5%). In addition, very few patients had 25-hydroxyvitamin D levels ≥75nmol/L (10.2%) which may have masked the hypothesized inverse relationship between 25-hydroxyvitamin D and HbA1c. 25-hydroxyvitamin D levels ≥75nmol/L has been selected by the Endocrine Society and vitamin D experts as the cut off point for vitamin D sufficiency beyond which non-calcemic functions of 25-hydroxyvitamin D may be observed (Holick et al., 2011).

Looking at the relationship between 25-hydroxyvitamin D and HbA1c by vitamin D status and by ethnicity, I found a negative association between 25-hydroxyvitamin D and HbA1c in Caucasians with 25-hydroxyvitamin D levels equal to or greater than 30nmom/L and less than 50nmol/L (n=31; β=-0.07, P=0.023). Also, there was a negative association between 25-hydroxyvitamin D and HbA1c in African Americans with 25-hydroxyvitamin D levels equal to or greater than 50nmom/L and less than 75nmol/L (n=25; β=-0.08, P=0.044). Despite these interesting findings, I cannot make definitive conclusions about the observed associations in both Caucasians and African Americans given the small sample size. However, these findings may be hypotheses generating and need to be tested in future studies.

Furthermore, although these finding were overall negative, this study identified 22.8% subjects with poor glycemic control, 48.2% subjects with intermediate glycemic

control, 40.6% subjects with vitamin D deficiency, and 49.2% subjects with vitamin D insufficiency. Poor glycemic control and vitamin D deficiency or insufficiency may be considered as prognostic biomarker in metabolically stable children and adolescents with T1DM. Poor glycemic control have been associated with the increased risk of developing micro- and macro-vascular complications such as nephropathy, neuropathy, retinopathy, and coronary and peripheral vascular disease, later in life (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group et al., 2009). Furthermore, severe vitamin D deficiency (25-hydroxyvitamin D levels less than 15.5nmom/L) has been identified as an independent predictor of all-cause mortality in adolescents and adults with T1DM (Joergensen et al., 2011).

In addition, these findings showed no correlation between 25-hydroxyvitamin D levels and BMI z-scores (P=0.486). Similarly, Svoren and colleagues observed no association between 25-hydroxyvitamin D and BMI z-scores in youth recently diagnosed with T1DM and youth with established T1DM (Svoren et al., 2009). However; these findings are contradictory to what have been reported in healthy, overweight, and obese children and adolescents (Kelly et al., 2011; Delvin et al., 2010; Johnson et al., 2010; Reis et al., 2009). The reason may be the unequal distribution of subjects across BMI percentiles (63.5% healthy weight; 23.3% overweight; and 13.2% obese) and the relatively low number of subjects stratified as obese.

Also, this is the first study to examine the relationship between 25-hydroxyvitamin D and blood glucose level in T1DM (P=0.195). This finding is contradictory to what has been previously reported in healthy, overweight, and obese

children and adolescents (Delvin et al., 2010; Johnson et al., 2010). One reason for the negative result may be the non-fasting blood glucose level used for this. Another reason may be the wide fluctuation of blood glucose level in individuals with T1DM. The glucose level used in this study was based on a single measurement and therefore may not reflect the true average of glucose level over the past week or even the past month.

***The Relationship between 25-hydroxyvitamin D and Inflammatory cytokines in Meta*bolically Stable Children and Adolescents with T1DM**

In animal studies, 25-hydroxyvitamin D has been established as an immune modulator that shifts the differentiation of CD4+ T cells towards a Th2, thereby decreasing the production of pro-inflammatory cytokines including IL-6 and up-regulating the production of anti-inflammatory cytokines including IL-10 (Bouillon et al., 2008; van Etten & Mathieu, 2005). Furthermore, the addition of vitamin D to activated monocytes from individuals with T1DM was shown to down-regulate the expression of TNF-α, IL-6, IL-1, and IL-8 (Giulietti et al., 2007). Also, the effect of vitamin D supplementations on inflammation has been reported in patients with end-stage renal disease, hip-fracture, chronic kidney disease, and congestive heart failure (Stubbs, Idiculla, Slusser, Menard, & Quarles, 2010; Miller et al., 2007; Neves et al., 2010; Schleithoff et al., 2006). In this sample of children and adolescents with T1DM, 25-hydroxyviatmin D was inversely associated with IL-6 (β=-0.007, P=0.030); however, this relationship was no longer significant after adjusting for socio-demographic and disease-related characteristics (β=-0.005, P=0.175). Similarly, I found no significant association between 25-hydroxyvitamin D and IL-10 in this sample (β=-0.002, P=0.729 and β=0.004, P=0.356; for unadjusted and adjusted models, respectively). Although these findings are

negative, they do not exclude the effect of vitamin D supplementation on inflammatory cytokines in T1DM. Intervention studies are needed to test these hypotheses.

The Relationship between HbA1c and Inflammatory cytokines in Metabolically Stable Children and Adolescents with T1DM

It is well established that hyperglycemia is linked to inflammation in T1DM (Erbagci et al., 2001; Foss-Freitas et al., 2008; Lo et al., 2004; Van Sickle et al., 2009; Zozulinska et al., 1999). I hypothesized that HbA1c is positively associated with IL-6 in metabolically stable children and adolescents with T1DM. In this sample, HbA1c was not associated with IL-6 even after adjusting for select socio-demographic and disease-related covariates (β=0.09, P=0.418). Interestingly, these findings are similar to the SEARCH case-control study where inflammation as evident by elevated levels of IL-6 was independent of glycemic control in adolescents and young adults with T1DM (10-22 years) (Snell-Bergeon et al., 2010).

An interesting finding was the positive association between anti-inflammatory cytokine IL-10 and HbA1c (β=0.21, P=0.008), adjusting for socio-demographic and disease-related covariates. A prospective study following newly diagnosed individuals with T1DM showed an association between higher mean number of IL-10 producing cells, measured at the time of T1DM diagnosis, and improved glycemic control (r^2=0.68 P=0.08) measured three months after diagnosis (Sanda, Roep, & von Herrath, 2008). Another study reported significantly elevated serum levels of IL-10 in T1DM children with ketoacidosis as compared to T1DM children with no ketoacidosis (P<0.01) (Dai et al., 2010). These study findings and those of others suggest that IL-10 is an independent

predictor of glycemic control in T1DM. IL-10 may be the target of immune modulatory gene therapy in this population (Goudy et al., 2003).

Other Findings

Glycemic Control in Metabolically Stable Children and Adolescents with T1DM

Poor glycemic control was found in 28.0% of the final sample. This number is higher than what has been previously reported (16.8%) in a nationally representative study of youth of T1DM (Petitti et al., 2009). The reason may be the high percent of African Americans with poor glycemic control (42.4%) represented in this study sample. Based on multivariate model; ethnicity, insurance, diabetes duration, type of insulin regimen, average total daily insulin dose, frequency of blood glucose monitoring per day, frequency of nurse practitioner clinic visit over the past year, and blood glucose level were all independent predictors of glycemic control. Although previous reports have shown age and sex to predict glycemic control this study failed to replicate these findings (Paris et al., 2009; Petitti et al., 2009; Levine et al., 2001). Reasons may be the relatively small sample size (n=197) and the truncated age range 7-18 years. Conversely, this study was similar to the SEARCH or Diabetes in Youth Study in that I found no association between BMI and HbA1c a relationship rather significant in youth with T2DM (Petitti et al., 2009). Furthermore, similar to published data, subjects on insulin pump had the lowest mean HbA1c (8.1% ± 1.1) as compared to subjects on MDI (8.4±1.2% to 8.7±1.4%) or mixed insulin twice per day (10.1±1.3%) (Paris et al., 2009).

Vitamin D Status in Metabolically Stable Children and Adolescents with T1DM

Based on functional studies of calcium absorption and parathyroid hormone in adults, serum 25-hydroxyvitamin D level equal to 75nmol/L has been selected as the cut

off point for vitamin D sufficiency (McKenna et al., 1998; Heaney et al., 2008). Despite the scarcity of research in children and adolescents; recently the Endocrine Society has adopted the same threshold for vitamin D sufficiency in this young population. Serum 25-hydroxyvitamin D levels less than 50nmol/L were considered deficient, and serum 25-hydroxyvitamin D levels equal to or greater than 50nmol/L or less than 75nmol/L were considered insufficient (Holick et al., 2011). For this study sample, 25-hydroxyvitamin D levels less than 75nmol/L were identified in 90.0% of study subjects; of which 40.6% were vitamin D deficient and 49.2% were vitamin D insufficient. This percent of subjects with 25-hydroxyvitamin D levels than 75nmol/L (90.0%) is higher than what has been previously reported for healthy and overweight youth 6-21 years (55.0%), and non-obese and obese youth 4-18 years (74.0%) recruited from the primary care practices at the Children's Hospital of Philadelphia (at latitude 40°N) (Kelly et al., 2011; Weng et al., 2007). It may be that the higher percent of vitamin D deficiency and insufficiency in this sample is due to the time of data collection from January to June when vitamin D cutaneous production is relatively low (Holick et al., 2007).

The IOM have challenged the current cut off point for vitamin D sufficiency and lowered the threshold from 75nmol/L to 50nmol/L based on studies of vitamin D and calcium. The IOM argues that the relationship between 25-hydroxyvitamin D and parathyroid hormone is not curvilinear and that peak calcium absorption occurs when 25-hydroxyvitamin D levels are between 50 and 75nmo/L (Rosen, 2011). The interpretation of my study findings in light of the current IOM recommendations can have significant clinical implications. According to the IOM classification of vitamin D status; 9.1% of subjects are at risk of deficiency, 31.5% of subjects are at risk of vitamin D inadequacy

and 59.4% are sufficient. Based on these cut off points, only 40.6% of study subjects will be advised vitamin D supplements. Whereas 90.0% of study subjects will be advised vitamin D supplements if clinical management was based on the Endocrine Society recommendations. Until there is evidence from well-powered studies linking serum 25-hydroxyvitamin D levels ≥75nmol/L to non-calcemic health outcomes, the cut off point for vitamin D sufficiency will remain controversial.

From the unadjusted model, 25-hydroxyvitamin D was significantly associated with age, sex, insurance status, type of insulin regimen, average total daily insulin dose, and pro-inflammatory cytokine IL-6. In a multivariate model controlling for age, sex, ethnicity, insurance status, BMI z-score, type of insulin regimen, average total daily insulin dose, diabetes duration, and pro-inflammatory cytokine IL-6; only age (β=-1.19; P=0.008), sex (β=5.13; P=0.042), type of insulin regimen (β=-3.33; P=0.007), and average total daily insulin dose (β=-9.39; P=0.018) were significantly associated with 25-hydroxyvitamin D. In another study examining these relationships in youth recently diagnosed with T1DM and youth with established T1DM; only age and ethnicity were significantly associated with 25-hydroxyvitamin D (Svoren et al., 2009). In healthy and overweight children and adolescents from the same latitude (40°N) as this study sample, 25-hydroxyvitamin D was significantly associated with vitamin D intake, race, and season (Weng et al., 2007). Also, in non-obese and obese children and adolescents at latitude 40°N, 25-hydroxyvitamin D was significantly associated with age, BMI, race, and season (Kelly et al., 2011). These findings suggest that race/ethnicity is a strong predictor of 25-hydroxyvitamin D in children and adolescents; however, this relationship did not hold true in this study sample (P=0.195).

Given the discrepancy between this study results and what was previously reported about the relationship between 25-hydroxyvitamin D and race/ethnicity, I re-examined this relationship in this study sample after excluding Hispanics and Others (n = 23). Furthermore, there was a notable difference in mean 25-hydroxyvitamin D across different racial/ethnic groups: 59.3±16.3nmoml/L for Caucasians, 45.0±15.6nmoml/L for African Americans, 55.9±21.4nmoml/L for Hispanics, and 68.7±15.0nmoml/L for Others.

In the unadjusted model, the relationship between 25-hydroxyvitamin D and ethnicity was significant (β=-14.28; P<0.000). Furthermore, after adjusting for age, sex, ethnicity, insurance status, BMI z-score, type of insulin regimen, average total daily insulin dose, diabetes duration, and pro-inflammatory cytokine IL-6; only age (β=-1.39; P=0.002), ethnicity (β=-13.20; P< 0.000), and average total daily insulin dose (β=-9.06; P= 0.018) were significantly associated with 25-hydroxyvitamin D. The findings on age and ethnicity are similar to what has been previously reported in youth with T1DM, and healthy, overweight, and obese youth at latitude 40°N. However, to my knowledge, the significant relationship observed between 25-hydroxyvitamin D and average total daily insulin dose is new. This relationship should be taken with caution since average total daily insulin dose was a self-reported measure and therefore may not reflect the true average insulin dose in this sample.

Inflammation in Metabolically Stable Children and Adolescents with T1DM

Pro-inflammatory Cytokine IL-8

Increased levels of inflammatory markers have been consistently reported in T1DM and further associated with diabetes-related micro-vascular complications (King,

2008). In this study sample of metabolically stable children and adolescents with T1DM, serum levels of pro-inflammatory cytokine IL-8 were not detected. These findings are not consistent with what has been previously reported. One study reported mean IL-8 of 3.7±4.0pg/ml in adolescents with HbA1c less than 9.0% and 7.4±4.3pg/ml in adolescents with HbA1c greater than 9.0% (Van Sickle et al., 2009). In the same study, serum levels of IL-8 were significantly associated with HbA1c (β=0.36, P=0.03). Another study reported elevated mean IL-8 (12.7±1.7pg/ml) in children with newly diagnosed T1DM and children with established T1DM as compared to non-diabetic controls (5.5±0.3pg/ml) (Erbagci et al., 2001). It is highly probable that I did not detect serum IL-8 levels in this study sample due to the low sensitivity of the R&D human CXCL8/IL-8 Immunoassay. The lowest standard for the R&D human CXCL8/IL-8 Immunoassay was 31.2pg/ml which may have been significantly higher than serum IL-8 levels present in this study sample.

Pro-inflammatory Cytokine IL-6

Elevated serum levels of IL-6 have been associated with acute and chronic hyperglycemia, onset of T1DM diagnosis, and diabetes-related micro-vascular complications (Erbagci et al., 2001; Rosa et al., 2008; Devaraj et al., 2007; Schram et al., 2005). Mean IL-6 was 1.1±1.2pg/ml for this study sample. These results are comparable to what has been previously reported in non-fasting children with T1DM ranging from 0.86±0.10pg/ml to 1.20±0.16pg/ml (Rosa et al., 2008). Interestingly, mean IL-6 level in this sample is significantly lower than what has been previously reported in glucose intolerant obese children and adolescents (2.7±1.1pg/ml) (Yeste et al., 2007). Also, these

data showed that serum IL-6 was significantly higher in females than males (1.3±1.4pg/ml versus 0.9±1.1pg/ml; respectively).

Table 5.1 Mean IL-6 of the final sample by BMI and by age

Normal weight	**7-12 years**	**13-18years**
Females	1.25±1.78	1.19±1.13
Males	0.83±0.85	0.96±1.08

Overweight/Obese	**7-12 years**	**13-18years**
Females	0.95±0.63	1.57±1.29
Males	1.26±1.86	0.59±0.31

After stratification of the sample by BMI and age; mean IL-6 was highest in overweight/obese adolescent females (1.57±1.29pg/ml) as compared to other subgroups (table 5.1). Furthermore, these results in normal weight (0.83±0.85pg/ml) and overweight/obese (1.26±1.86pg/ml) males 7-12 years are comparable to IL-6 levels reported in normal weight (1.3pg/ml; range 0 to 2.5pg/ml), and overweight/obese (1.4pg/ml; range 0.2 to 3.6pg/ml) boys aged 8 years (Charmaine et al., 2010). Conversely, these results in normal weight (0.96±1.08pg/ml) and overweight/obese (0.59±0.31pg/ml) adolescent males are much lower than what have been reported in normal weight (7.9pg/ml; range zero to 9.2pg/ml), and overweight/obese (8.5pg/ml; range 7.1 to 10.4pg/ml) adolescent males aged 15 years (Charmaine et al., 2010).

In essence, overweight/obese females (mean IL-6; 1.57±1.29pg/ml) may be at a higher risk of developing diabetes-related micro-vascular complications as compared to overweight/obese males within the same age group (mean IL-6; 0.59±0.31pg/ml), and overweight/obese males and females 7-12 years (mean IL-6; 1.26±1.86pg/ml and 0.95±0.63pg/ml, respectively).

Anti-inflammatory Cytokine IL-10

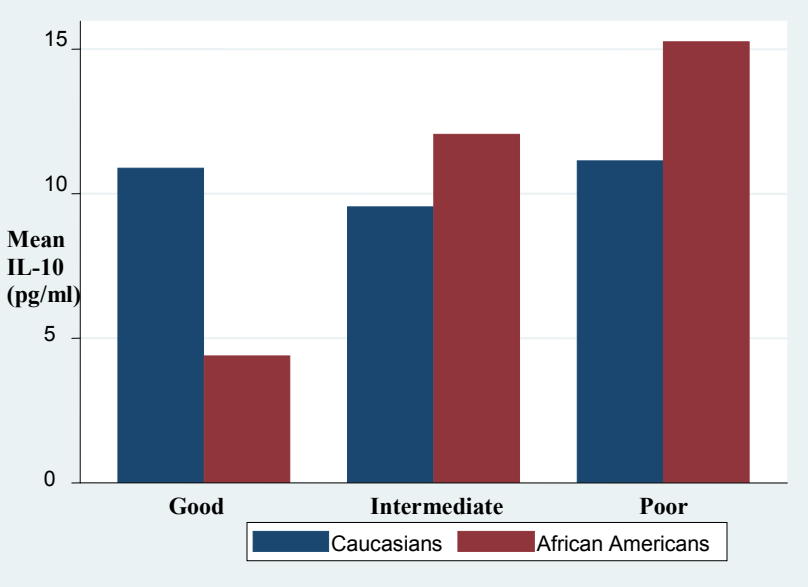
Mean anti-inflammatory cytokine IL-10 was 10.5±20.3pg/ml in this study sample of metabolically stable children and adolescents with T1DM. These findings are comparable to mean IL-10 levels (ranged from 5.4 to 7.6pg/ml) measured at baseline and at short time intervals during exercise for normal weight T1DM children 13.9±0.3years (Rosa et al., 2011). Also, mean IL-10 levels in this sample were higher than what has been reported for normal weight (0.6pg/ml; range 0.2 to 0.9pg/ml) and overweight/obese (0.6pg/ml; range 0.03 to 1.1pg/ml) boys aged 8 years, and for normal weight (7.7pg/ml; range 0.9 to 7.9pg/ml) and overweight/obese (7.9pg/ml; range 7.3 to 8.1pg/ml) adolescent males aged 15years (Charmaine et al., 2010).

The moderately elevated levels of serum IL-10 in this study sample is not an unlikely finding given the chronic state of inflammations associated with T1DM. IL-10 is known to inhibit Th1 responses, inhibit pro-inflammatory cytokines, and increase the expression of Foxp3+ in T cells, thus changing their phenotype into T regulatory cells. Furthermore, studies have shown that vitamin D enhances the development of IL-10 producing cells which then reduces the number of IL-6 producing cells (Correale, Ysrraelit, & Gaitan, 2009). In bivariate analysis, serum IL-10 was positively correlated to IL-6 (P=0.037); however, the relationship was no longer significant after adjusting for age, sex, ethnicity, BMI, and HbA1c.

Furthermore, mean IL-10 was significantly correlated to HbA1c (P=0.015); however, after stratifying the sample by race/ethnicity, this relationship was no longer significant for Caucasians (P=0.695) but remained significant for African Americans (P=0.006). Also, serum IL-10 levels were not significantly different across levels of

glycemic control for Caucasians (P=0.712) but showed a trend towards significance for African Americans (P=0.085) (figure 5.1). The significant correlation observed between HbA1c and IL-10 in African Americans is most likely due to the high percentage of African Americans with poor glycemic control in this study sample. Furthermore, these data suggest that the relationship between HbA1c and IL-10 in this sample is not linear. A threshold value for HbA1c beyond which increases in serum levels of IL-10 are expected should be determined. In this sample, this may not be feasible since I have excluded all patients with HbA1c greater than 12.0%.

Figure 5.1 Mean IL-10 of the final sample by ethnicity and by glycemic control



Limitations of the Study

The main limitation of this study is its cross-sectional design, and therefore the causative nature of observed significant associations could not be established. In addition, a larger and more ethnically diverse sample may be needed to examine whether the relationships observed vary by ethnicity. Moreover, this study was based on a single

blood draw to measure 25-hydroxyvitamin D, inflammatory cytokines, blood glucose level, and HbA1c and therefore, it does not take into account within-person variation. Furthermore, covariates including vitamin D supplementation, diet, exercise, adherence, and family diabetes-management that may explain a significant percent of variance in HbA1c and/or 25-hydroxyvitamn D were not included in this study. Lastly, the study sample was recruited at a single site, and therefore the results of this study may not be generalizable.

Conclusions and Clinical Implications

The knowledge gained from the study has increased our understanding on the relationships among vitamin D status, glycemic control, and inflammation in metabolically stable children and adolescents with T1DM. This study confirmed what is already known on the marked prevalence of vitamin D deficiency in children and adolescents with T1DM. Also, this study showed that a significant number of children and adolescents with T1DM have poor glycemic control. These findings are alarming given that this study sample includes T1DM individuals motivated to volunteer for research and attends a specialty diabetes center.

In this study sample, 25-hydroxyvitamin D was not associated with HbA1c. Despite this negative finding, clinical controlled trials are still needed to assess the short-term and long-term effect of varied doses of vitamin D supplements on HbA1c in children and adolescents with T1DM. The possibility that vitamin D may be involved in glucose homeostasis in T1DM has potential implications for a) establishing vitamin D testing as “standard of care” in T1DM, and b) recommendations regarding the use of vitamin D supplements and sun exposure in T1DM.

Until there is sufficient evidence to support the inverse relationship between 25-hydroxyvitamin D and HbA1c in T1DM, the findings of this study may be used to highlight the significantly high prevalence of vitamin D deficiency and insufficiency in children and adolescents with T1DM. Future studies assessing the prevalence of vitamin D deficiency and insufficiency across the full spectrum of HbA1c are needed. In addition, since this study enrolled patients from January through June, it is important to assess vitamin D status in children and adolescents with T1DM across four seasons (fall, winter, spring, and summer). It is also of relevance to look into the effect of vitamin D deficiency and insufficiency on bone health in T1DM.

Mean serum IL-6 was highest in overweight/obese females (1.57±1.29pg/ml). Mean serum IL-10 was highest in African American subjects with poor glycemic control (15.2±22.5pg/ml). Serum levels of pro-inflammatory cytokine IL-8 were not detected in this study sample. Pro-inflammatory cytokine IL-6 was not associated with HbA1c. Conversely, anti-inflammatory cytokine IL-10 was positively associated with HbA1c (β=0.12, P=0.039). These findings call for a special attention to overweight/obese females with T1DM since high IL-6 levels have been linked to the development of micro- and macro-vascular complications in this population. Furthermore, anti-inflammatory cytokine IL-10 may be a potential prognostic biomarker for poor glycemic control in this population. Unfortunately, I did not find a significant association between 25-hydroxyvitamin D, IL-6 and IL-10 in this study sample. Physiologically, poor glycemic control is associated with elevated levels of pro-inflammatory cytokines. In addition, it is well established that elevated levels of anti-inflammatory cytokine IL-10 down-regulate the production of pro-inflammatory cytokines including IL-6 *in vivo* and *in vitro*. Studies

have also shown that vitamin D enhances the development of IL-10 producing cells *in vivo* and *in vitro*. In essence, intervention studies are needed to assess the effect of varied doses of vitamin D supplements on pro- and anti-inflammatory cytokines and ultimately measure the effect of inflammatory cytokines on HbA1c in T1DM.

APPENDIX

The Children's Hospital of Philadelphia®

INFORMED CONSENT FORM AND HIPAA AUTHORIZATION

Study Title: The Relationship between Vitamin D Status and Glycemic Control in Children and Adolescents with Type 1 Diabetes Mellitus: Role of Inflammatory Mediators

IRB #: 10-007770

Version Date: October 18, 2010 - Version 2

Principal Investigator: Terri H Lipman, PhD, CRNP, FAAN — Telephone: (215) 590-6587, (215) 898-2259

Affiliations: The Children's Hospital of Philadelphia, Division of Endocrinology

Study Sponsor: Office of Nursing Research at the University of Pennsylvania, School of Nursing

You may be eligible to take part in a research study. This form gives you important information about the study. It describes the purpose of this research study, and the risks and possible benefits of participating.

If there is anything in this form you do not understand, please ask questions. Please take your time. You do not have to take part in this study if you do not want to. If you take part, you can leave the study at any time.

Parents or legal guardians, who are giving permission for a child, please note: in the sections that follow the word 'you' refers to your child.

Why are you being asked to take part in this study?

You are being invited to take part in this research study because you have had type 1 diabetes mellitus for more than one year, your age is between 7 and 18 years, and you may be doing your yearly screening blood tests today.

What is the purpose of this research study?

The purpose of this research study is to find out whether vitamin D level is related to blood glucose level in children and adolescents with type 1 diabetes mellitus. Previous studies have shown that keeping vitamin D levels within the normal range is important for bone growth and development, and may be helpful in blood glucose control.

The study will also look at how inflammation affects the relationship between vitamin D level and blood glucose level in young people with type 1 diabetes.

How many people will take part?

About 204 participants will take part in the study. All participants will be children or adolescents with type 1 diabetes mellitus, who have had diabetes longer than one year, and are being followed at the Diabetes Center for Children (DCC) at the Children's Hospital of Philadelphia (CHOP).

What is involved in the study?

If you agree to take part in this study, the following procedures will be performed:

Medical Record Review: We will review your medical record to collect some demographic information (e.g. age, sex, ethnicity, and insurance) as well as information about your blood glucose control (HbA1c) and other information about your diabetes.

Blood Test: We will collect two teaspoons of your blood to measure vitamin D level and markers of inflammation at the same time you are having blood drawn for your regular yearly blood tests. If the blood is being drawn at CHOP today, you will NOT need an extra needle stick for the two teaspoons of blood that will be collected for this study.

If you go to an outside lab for blood drawing you may still participate in the study. However, this would require an extra needle stick for the two teaspoon of blood in the CHOP laboratory.

Your Vitamin D levels will be shared with your diabetes provider. If the results are abnormal, your diabetes provider may check your vitamin D level one more time at a local laboratory.

Your participation will end once you give two teaspoons of blood.

Visit Schedule

The table below provides a brief description of the purpose and duration of each study visit.

Visit	Purpose	Procedures	Duration
Visit 1 (only visit)	Screening and enrollment visit	Blood test	1 hour

What are the risks of this study?

Taking part in a research study involves inconveniences and risks. If you have any questions about any of the possible risks listed below, you should talk to the study investigators Terri Lipman, PhD, CRNP, FAAN and Sarah Sawah, PhD(c), MSc, RN. You can also talk to your regular nurse practitioner or doctor.

Taking blood may cause some pain, bleeding or bruising at the spot where the needle enters your body. Rarely, taking blood may cause fainting or infection. As stated above, we will take your blood at the same time you are having blood drawn for clinical purposes. The amount of blood we will take is minimal and you will not need a separate needle stick.

If you go to an outside lab for blood draw and you wish to participate in this study, you will have to undergo an additional blood draw procedure at CHOP laboratory. The risks of taking blood are the same as stated above.

As with any study involving collection of data, there is the possibility of loss of confidentiality of data. Every precaution will be taken to secure participants' personal information to ensure confidentiality.

At the time of participation, each participant will be assigned a study identification number. This number will be used on data collection forms, on your blood sample and in the study database instead of names and other private information. A separate list will be maintained that will link each participant's name to the study identification number for future reference and communication.

Are there any benefits to taking part in this study?

There will be no direct benefit to you from taking part in this study. We hope that the information learned from this study will help us understand more about type 1 diabetes and possibly benefit other children and adolescents with the same condition in the future. If the results of the study show that vitamin D is related to blood glucose levels, the results may encourage diabetes providers to include vitamin D in yearly screening tests.

Do you need to give your consent in order to participate?

Once you read this form and have your questions answered, you will be asked to decide if you wish to participate. If you wish to participate in this study, you must sign this form. A copy will be given to you to keep as a record.

What happens if you decide not to take part in this study?

Participation in this study is voluntary; you do not have to take part in order to receive care at CHOP. If you decide not take part or if you change your mind there will be no penalties or loss of any benefits to which you are otherwise entitled. Your current and future medical care at CHOP will not be affected by your decision.

What about privacy, authorization for use of Personal Health Information (PHI) and confidentiality?

We need to collect health information about you in order to conduct this study. This includes information about you from medical records and from the tests that are part of this research. Routine clinical laboratory tests performed as part of this study will appear in your medical record. We will do our best to keep your personal information private and confidential. However, we cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

The results of this study may be shown at meetings or published in journals to inform other doctors and health professionals. We will keep your identity private in any publication or presentation about the study.

People and organizations that may inspect and/or copy your research records to conduct this research, assure the quality of the data and to analyze the data include:

Members of the research team at CHOP and UPenn;

Medical staff who are directly or indirectly involved in your care related to this research;

People who oversee or evaluate research and care activities at CHOP;

People from agencies and organizations that perform independent accreditation and/or oversight of research; such as the Department of Health and Human Services, Office for Human Research Protections.

By law, CHOP is required to protect your health information. The research staff will only allow access to your health information to the groups listed above. By signing this document, you are authorizing CHOP to use and/or release your health information for this research. Some of the organizations listed above may not be required to protect your information under Federal privacy laws. If permitted by law, they may be allowed to share it with others without your permission.

There is no set time for destroying the information that will be collected for this study. Your permission to use and share the information and data from this study will continue until the research study ends and will not expire. Researchers continue to analyze data for many years and it is not possible to know when they will be completely done.

What are my rights and responsibilities as a research subject?

Taking part in a research study involves time and responsibilities. Please consider the study time commitments and responsibilities as a research subject when making your decision about participating in this study.

You may change your mind and take back your authorization to use and disclose your health information at any time. Even if you take back your authorization, we may still use and disclose the health information we have already obtained about you as necessary to maintain the integrity or reliability of the current research. To take back your authorization, you must send a letter to Terri Lipman, PhD, CRNP, who is also a nurse practitioner in the Diabetes Center. In the letter, you must say that you changed your mind and do not want us to collect any more health information about you. If you ask that we no longer collect your health information you will have to leave the study.

Financial Information

Will you be paid for taking part in this study?

You will be given a $5 gift card as a token of appreciation for taking part in this study.

Who is funding this research study?

The Office of Nursing Research at the University of Pennsylvania, School of Nursing and the Pediatric Endocrine Nursing Society are providing funding for this study.

Please ask Terri Lipman, PhD, CRNP, FAAN or Sarah Sawah, PhD(c), MSc, RN if you have any questions about how this study is funded.

What if you have questions about the study?

If you have questions about the study, call the study investigator Terri Lipman, PhD, CRNP, FAAN at 215-590-6587 or Sarah Sawah, PhD(c), MSc, RN at 215-909-0833. You may also talk to your own nurse practitioner or doctor if you have questions or concerns.

The Institutional Review Board (IRB) at The Children's Hospital of Philadelphia has reviewed and approved this study. The IRB looks at research studies like these and makes sure your rights and welfare are protected. You can talk to a person from this group if you have questions about your rights as someone taking part in a research study. You can call the IRB Office at 215-590-2830 if you have questions or complaints about the study.

Consent to Take Part in this Research Study and Authorization to Disclose Health Information

The research study and consent form have been explained to you by:

Person Obtaining Consent	Signature of Person Obtaining Consent Date:

By signing this form, you are indicating that you have had your questions answered, you agree to take part in this research study and you are legally authorized to consent to your child's participation. You are also agreeing to let CHOP use and share your health information as explained above. If you don't agree to our collecting, using and sharing your health information, you cannot participate in this study. **NOTE:** *A foster parent is not legally authorized to consent for a foster child's participation.*

Name of Subject

Date

Name of Authorized Representative (if different than subject)	Relation to subject: ☐ Parent ☐ Legal Guardian
Signature of Authorized Representative	Date

Child Assent to Take Part in this Research Study

For children capable of providing assent:

I have explained this study and the procedures involved to _____ in terms he/she could understand and that he/she freely assented to take part in this study.

_____
Person Obtaining Assent

_____ _____
Signature of Person Obtaining Assent Date

This study has been explained to me and I agree to take part.

_____ _____
Signature of Subject (optional) Date

For children unable to assent:

I certify that _____ was not capable of understanding the procedures involved in the study sufficiently to assent to study participation.

_____
Person Responsible for Obtaining Assent

_____ _____
Signature of Person Responsible Date

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