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Vitamin D levels, vitamin D receptor polymorphisms and HOMA2 model in Cuban Americans, Haitian Americans, and African Americans with and without type 2 Diabetes

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

VITAMIN D LEVELS, VITAMIN D RECEPTOR POLYMORPHISMS AND HOMA2
MODEL IN CUBAN AMERICANS, HAITIAN AMERICANS, AND AFRICAN
AMERICANS WITH AND WITHOUT TYPE 2 DIABETES.

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

DIETETICS AND NUTRITION

by

Lamya H. Shaban

2012

To: Interim Dean Michele Ciccazzo
Robert Stempel College of Public Health and Social Work

This dissertation, written by Lamya H. Shaban, and entitled Vitamin D levels, vitamin D receptor polymorphisms and HOMA2 model in Cuban Americans, Haitian Americans, and African Americans with and without type 2 Diabetes, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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DEDICATION

I dedicate this dissertation to my father and mother, for without your stability and support throughout my life I wouldn't have made it this far.

To my family in Kuwait, I thank you for all your trips to see me, support and encourage me. To my husband who gave me his unconditional support, and who has shown me that I am more than I think I can be. To my two beautiful God sent children whose time was stolen from them in order for me to attain a Ph.D. and to whom I shall forever be indebted.

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ABSTRACT OF THE DISSERTATION

VITAMIN D LEVELS, VITAMIN D RECEPTOR POLYMORPHISMS AND HOMA2
MODEL IN CUBAN AMERICANS, HAITIAN AMERICANS, AND AFRICAN
AMERICANS WITH AND WITHOUT TYPE 2 DIABETES.

by

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Florida International University, 2012

Miami, Florida

Professor Fatma G. Huffman, Major Professor

This cross sectional study investigated the association between 25-hydroxyvitamin D (25(OH)D) levels, vitamin D receptor (VDR) polymorphisms, HOMA2 and diabetes status in Cuban Americans, Haitian Americans, and African Americans. The sample for the study included a total of 885 participants (Cuban American = 370; Haitian American = 259; African American = 256). Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping *BsmI* and *TaqI*, Real-time- Polymerase chain reaction (RT-PCR) was used for *Apal*. HOMA2 model calculations were used as a surrogate marker for insulin resistance, insulin sensitivity and β -cell function. All statistical analyses were performed using SPSS (Version 18.0, Chicago, IL, U.S.). Student's *t*-test and analysis of variance (ANOVA), χ^2 test and logistic regression analysis were used. We found that Cuban Americans without T2D had significantly lower odds of having insufficient 25(OH)D compared to all other groups. Participants with darker skin (Haitian

Americans and African Americans) and those with T2D had the greatest risk of having insufficient levels of 25(OH)D. Cuban Americans with T2D had a protective factor for vitamin D insufficiency if they carried the *TaqI* genotype (tt) ($p < 0.02$) and Cuban Americans without T2D had the highest β -cell function levels ($p < 0.05$).

Further investigation is needed to have a better understanding of the role vitamin D, VDR polymorphisms and the role HOMA2 model plays in the three ethnicities. Awareness of the high prevalence of vitamin D insufficiency among Haitian Americans and African Americans and also in those with T2D may sensitize physicians and dietitians to increase efforts to prevent vitamin D insufficiency. Further research to investigate the role and mechanism of action of vitamin D and diabetes is warranted.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
Specific aims and hypotheses	3
II. REVIEW OF LITERATURE.....	7
Sources of vitamin D	8
Synthesis and metabolism of vitamin D	10
Mechanism of vitamin D action.....	13
Vitamin D, insulin secretion, sensitivity and resistance	15
Vitamin D and type 2 diabetes.....	17
Longitudinal/Cohort Studies.....	17
Randomized controlled/Cross-sectional studies	19
Vitamin D and sun exposure in the Sunshine State	20
Vitamin D deficiency in the elderly.....	21
Vitamin D receptor	22
VDR polymorphism and insulin secretion.....	22
VDR polymorphism and insulin resistance	23
VDR polymorphism and type 2 diabetes	25
III. SERUM VITAMIN D INSUFFICIENCY AND DIABETES STATUS IN THREE ETHNIC GROUPS.....	28
IV. VITAMIN D RECEPTOR POLYMORPHISMS, VITAMIN D LEVELS, AND DIABETES STATUS IN THREE ETHNIC GROUPS.....	50
V. HOMA2 MODEL, VITAMIN D LEVELS AND DIABETES STATUS IN THREE ETHNIC GROUPS.....	73
VI. SUMMARY AND DISCUSSION	91
VII. STRENGTHS AND LIMITATIONS	95
VIII. FUTURE RESEARCH.....	96
REFERENCES	97
APPENDIX.....	110
VITA.....	112

LIST OF TABLES

TABLE	PAGE
 CHAPTER III.	
Table 1. Characteristics of study participants by ethnicity	46
Table 2. Characteristics of study participants by diabetes status.....	47
Table 3. Characteristics of study participants by 25-hydroxyvitamin D status	48
Table 4. Odds of having insufficient 25-hydroxyvitamin D by diabetes status and ethnicity.....	49
 CHAPTER IV.	
Table 1. Characteristics of study participants by diabetes status.....	69
Table 2. Characteristics of study participants by vitam D receptor genotypes	70
Table 3. Characteristics of study participants within three ethnicities by vitamin D status	71
 CHAPTER V.	
Table 1. Characteristics of study participants by diabetes status.....	87
Table 2. Characteristics of study participants without and with type 2 diabetes by vitamin D status	88
Table 3. Characteristics of study participants without and with type 2 diabetes by ethnicity.....	89
Table 4. HOMA2- β means by vitamin D status and ethnicity within diabetes status	90
 APPENDIX.	
Table 1. Vitamin D receptor genotype (<i>BsmI</i>) in participants by diabetes status.....	110

I. INTRODUCTION

Type 2 diabetes (T2D) accounts for about 90 to 95 percent of all diagnosed cases of diabetes (ADA, 2011). The physiology of the disease begins with insulin resistance where the cells of the body become resistant and do not use insulin properly. In turn the body produces greater amounts of insulin and as the need for insulin rises, the pancreas gradually loses its ability to produce insulin in sufficient quantities (NIDDK, 2008). Type 2 diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity (NIDDK, 2008). African Americans, Hispanic/Latino Americans, American Indians, and some Asian Americans and Native Hawaiians or other Pacific Islanders have been reported to be at high risk for type 2 diabetes and its complications (NIDDK, 2008). In addition to other lifestyle factors, there is a growing interest in the association between ethnicity, T2D and vitamin D, in particular, vitamin D insufficiency and Vitamin D Receptor (VDR) polymorphisms.

Vitamin D deficiency and its relationship to impaired glucose tolerance along with the discovery of receptors of 1, 25-dihydroxyvitamin D₃ (the active form of vitamin D) have created interest in the potential role of vitamin D in the pathogenesis of the disease (Mathieu, Gysemans, Bouillon, & Bouillon, 2005). Since the early 1980s it has been reported that vitamin D deficiency inhibits pancreatic secretion and turnover of insulin, leading to impaired glucose metabolism (Mathieu et al., 2005). It was thought that vitamin D deficiency in fact hinders insulin secretion (Boucher, 1998; Norman, Frankel, Heldt, & Grodsky, 1980) through β - cell dysfunction (Chiu, Chu, Go, & Saad, 2004) and

modulates lipolysis (Diririzo, & Zemel, 2000; Rammos, Tseke, & Ziakka, 2008; Zemel, Shi, Greer).

Although it is not exactly clear how the pathogenesis of hypovitaminosis D could affect glucose/insulin metabolism, a number of studies indicated an association of low serum vitamin D levels with high serum glucose and insulin resistance (Choi, 2011; Gannagé-Yared, 2009; Reis, 2009). However, there is no documented evidence to date regarding vitamin D, T2D and clinical outcomes comparing ethnic groups such as Cuban Americans, Haitian Americans, and African Americans. Along with the relationship of vitamin D with insulin secretion, the vitamin D receptor (VDR) gene may also play a role in T2D, insulin secretion and insulin resistance. VDR polymorphisms, vitamin D and their association with T2D is a novel field of research. The interaction between the genetic variations between different ethnicities, i.e., Cuban Americans, Haitian Americans, and African Americans and the role vitamin D plays in T2D has not been explored.

The proposed study is an ancillary study that will use existing blood samples, dietary and other data from the parent study to generate new data on understudied ethnic groups (Cuban Americans, Haitian Americans, and African Americans). Blood samples will be used to generate data in order to assess the association between vitamin D status, diabetes status, vitamin D receptor polymorphisms and the HOMA2 model.

The proposed project will consist of three chapters; each chapter will address specific aims and hypotheses.

Specific aims and hypotheses:

**CHAPTER III. SERUM VITAMIN D INSUFFICIENCY AND DIABETES
STATUS IN THREE ETHNIC GROUPS**

Specific Aim 1: To determine the association between vitamin D status and diabetes status in Cuban Americans, Haitian Americans, and African Americans.

Hypothesis 1a: Cuban American, Haitian American, and African American participants with T2D will have lower serum vitamin D levels than their counterparts without T2D.

- Vitamin D status was measured through serum 25(OH)D using a sandwich based Enzyme-Linked ImmunoSorbant Assay (ELISA kit). Glycemic control was determined by measuring A1c with a monoclonal method. Existing research has found that persons with adult-onset diabetes have low levels of vitamin D compared to their counterparts without diabetes (Cavalier, 2011; Isaia, Giorgini, & Adami, 2001; Pilz, 2011; Scragg et al., 1995).

Hypothesis 1b: Haitian American and African American participants will have lower serum vitamin D levels as compared to their Cuban American counterparts in both participants with and without T2D.

- Serum Vitamin D status was measured as in Hypothesis 1a. Due to the fairer skin of the Cuban Americans it was assumed that they will have more efficient conversion of vitamin D by UVB rays (duration of sun exposure was not measured).

Hypothesis 1c: Cuban Americans, Haitian Americans, and African Americans with adequate (> 400 IU/day) vitamin D intake will have ‘sufficient’ (≥ 75 nmol/L) serum vitamin D levels regardless of diabetes status.

- Vitamin D intake was measured through a semi-quantitative food frequency questionnaire developed by Walter C. Willett (Willett, Sampson, & Stampfer, 1985). Hypothesis 1a outlines serum Vitamin D measurements.

Chapter IV. VITAMIN D RECEPTOR POLYMORPHISMS, VITAMIN D LEVELS, AND DIABETES STATUS IN THREE ETHNIC GROUPS

Specific Aim 1: To determine the association of vitamin D receptor (VDR) polymorphisms (*BsmI*, *TaqI* and *ApaI*), and diabetes status in Cuban Americans, Haitian Americans, and African Americans.

Hypothesis 1a: There will be a higher prevalence of polymorphisms in the VDR gene restriction sites *BsmI*, *TaqI* and *ApaI* for participants with T2D as compared to participants without T2D for all ethnicities.

- VDR polymorphisms will be determined using restriction enzymes *BsmI*, *TaqI* and *ApaI*. Several studies indicate that polymorphisms are present in individuals with T2D (Bid, Konwar, & Aggarwal, 2009; Malecki, Frey, & Moczulski, 2003; Ye, Reis, & Dubois-Laforgue, 2001), however no ethnic differences have been studied.

Hypothesis 1b: There will be differences in the prevalence and types of VDR polymorphisms among the three ethnicities in participants with and without T2D.

- Hypothesis 1a details methodology for VDR polymorphism determinations.

CHAPTER V. HOMA2 MODEL, VITAMIN D LEVELS AND DIABETES STATUS IN THREE ETHNIC GROUPS

Specific Aim 1: To determine the association between HOMA2 model, vitamin D, and diabetes status in Cuban Americans, Haitian Americans, and African Americans.

Hypothesis 1a: Insulin resistance (HOMA2) will be negatively associated with serum vitamin D levels for participants with and without T2D for all ethnicities.

- Insulin secretion will be measured using an ELISA kit. Homeostasis model assessment (HOMA2) score will be calculated using insulin secretion and fasting plasma glucose. HOMA2 is a method used to assess β -cell function, insulin resistance and insulin sensitivity. Vitamin D levels will be measured through serum 25(OH)D.

Hypothesis 1b: β -cell function will be positively associated with serum vitamin D levels for participants with and without T2D for all ethnicities.

- β -cell function will be calculated using insulin secretion and fasting plasma glucose. Vitamin D status will be measured through serum 25(OH)D using a sandwich based Enzyme-Linked ImmunoSorbant Assay (ELISA kit).

Additional Hypotheses tested:

Hypothesis 1a: Cuban Americans, Haitian Americans, and African Americans with each polymorphism (*BsmI*, *TaqI* and *ApaI*) will have greater insulin resistance than those without VDR polymorphisms regardless of diabetes status and ethnicity.

- Insulin resistance will be calculated using the HOMA2 model through the use of the insulin secretion and fasting plasma glucose.

Hypothesis 1b: Cuban Americans, Haitian Americans, and African Americans with each polymorphism (*BsmI*, *TaqI* and *ApaI*) and ‘insufficient’ serum vitamin D levels (less than 75nmol/L) will have lower insulin secretion than those with adequate serum vitamin D levels regardless of diabetes status.

- Serum vitamin D levels will be assessed using a commercial ELISA kit and insulin secretion will be measured using a sandwich ELISA assay. It has been indicated that vitamin D and its receptor complex may play a role in β - cell insulin secretion.

Hypothesis 1c: Cuban Americans, Haitian Americans, and African Americans with T2D and VDR polymorphisms (*BsmI*, *TaqI* and *ApaI*) will have lower insulin secretion than those without polymorphisms.

II. REVIEW OF LITERATURE

According to the National Center for Chronic Disease Prevention and Health Promotion (CDC) age adjusted data indicated that minority populations are disproportionately affected by diabetes, with Black males and females having the highest percent of diabetes in 2004, with Hispanics closely behind them. Although the rate of diabetes has increased generally in all ethnic groups from 1980 through 2005, the age-adjusted prevalence of diagnosed diabetes doubled among Black males and increased by 69% among Black females. However, of all groups observed, Black females had the highest overall prevalence (CDC, 2009).

Based on the National Health and Nutrition Examination Survey I (NHANES I) and its four follow-up surveys, adults with diabetes had a significantly higher risk of death, lower survival, and a lower quality of life compared with adults without diabetes (Gu, Cowie, & Harris, 1998). Most of these deaths were due to diabetes itself or its accompanying complications. The four leading causes of death among persons with T2D were: a) Cardiovascular disease (CVD) (~50%); b) diabetes itself (13%); c) malignant neoplasm (13%); and d) stroke (10%) (Harris, 1995). Less than half of the people (~ 45%) with T2D have adequate glycemic control (A1c levels of less than 7% which is the goal for persons with diabetes). Non-Hispanic Black women, Mexican American and African American men, ranked disproportionately higher for poor glycemic control (A1c less than 8%) (Harris, 1995).

According to U.S. death certificates in 2006 diabetes was the seventh leading cause of death (NIDDK, 2008). More recently, age-adjusted 2004-2006 national survey data showed that 3.7 million African Americans had T2D; 14.7 percent of all non-

Hispanic Blacks aged twenty and older had diagnosed and undiagnosed diabetes and 11.8 percent had diagnosed diabetes (CDC, 2008). After adjusting for age differences, the 2007–2009 national survey data for people aged 20 years or older indicated that 7.1% of non-Hispanic Whites, 11.8% of Hispanics (7.6% for Cubans) and 12.6% of non-Hispanic Blacks had diagnosed diabetes. Compared to White adults, the risk of diagnosed diabetes was 66% higher among Hispanics, and 77% higher among non-Hispanic Blacks (CDC, 2011).

There is limited information and statistics for African Americans, Haitian Americans and Cuban Americans as separate minority sub- groups with regard to diabetes. The existing few findings on African Americans and Hispanics; however, reflect the need to assess underlying factors for this increase and possible triggers or ethnic differences that may be a promoting factor.

Sources of vitamin D

Vitamin D refers to the secosterols; specifically, ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Vitamin D₂ is ingested through commercial irradiation of plant sterols (ergosterol). Vitamin D₃ can either be formed: endogenously by the skin after sun exposure (UVB solar wavelengths 290-320 nm) or through consumption of food of animal origin (Mathieu et al., 2005; Webb, Pilbeam, Hanafin, & Holick, 1990). The few dietary sources of vitamin D are somewhat limited and restricted to foods of animal origin such as fatty fish; cod liver oil (400 IU /tsp; and egg yolks (approx 20 IU) (Holick, 2002; Holick, 2006). Solar UVB wavelength irradiation is the primary source of vitamin D for most people (other than through dietary supplementation) (Holick, 2002, 2004a,

2004b); however, some factors such as race; ethnicity; and age play a role in the amount of UVB waves that can enter into the skin and the rate at which vitamin D can be synthesized. Because of the limited amount of sunlight exposure and different skin pigmentation (dark and light- skinned) the synthesis of vitamin D from sun exposure tends to be inadequate and does not meet requirements of circulating 25-hydroxyvitamin D₃ (25(OH)D) concentrations of 50-150nm/L. Most advantageous serum concentrations of 25(OH)D begin at 75 nmol/L (30 ng/mL) (Bischoff-Ferrari et al., 2006). Therefore, some countries such as the United States have fortified foods in an attempt to increase vitamin D levels. Foods such as milk (100 IU/8oz); some cereals (100 IU/serving); some yogurts (100 IU/serving); orange juice (100 IU/8oz); and margarine have been fortified. The estimated average requirement of vitamin D per day should be greater than 400 IU/day or greater than 10 µg per day (IOM, 2010). Persons on special diets such as those on macrobiotic diets (Fitzpatrick et al. 2000), vegetarians (Kaper et al., 2000), strict vegans (Dunn-Emke et al., 2005) and those consuming unfortified milk alternatives such as soy and almond milk may be at greater risk of vitamin D deficiency if not exposed to adequate UVB rays (Carvalho, 2001). Recommended dietary allowance of vitamin D have been defined by the Institute of Medicine (IOM) as 600 IU/day for those between the ages of 1-70 (including pregnancy and lactation), and 800 IU/day in those over 70. The upper tolerable level has been defined as 1,000 IU for infants 0-6 months, 1,500 IU for infants 6-12 months, 2,500 IU for children 1-3 years, 3,000 IU for 4-8 years and 4,000 IU for anyone older than eight years of age. This upper limit represents a safe upper boundary of the scale, however IOM stresses it is not what one should strive to consume (IOM, 2010).

Synthesis and metabolism of vitamin D

After vitamin D is obtained from sun exposure, food, and dietary supplements it is biologically inert and must undergo two hydroxylations in the body for activation. The first occurs in the liver where vitamin D is converted into 25-hydroxyvitamin D (25(OH)D), also known as calcidiol. The second hydroxylation occurs primarily in the kidneys where it is enzymatically converted to the physiologically active 1,25-dihydroxyvitamin D (calcitriol) (Figure 1). Although serum 1,25-dihydroxyvitamin D (1,25(OH)₂D) is the biologically active hormone form of vitamin D, the serum 25(OH)D is the most reliable indicator (in individuals without kidney disease) of vitamin D status, because it has a longer biological half-life than 1,25(OH)₂D, and circulates in much higher concentrations. Serum 25(OH)D reflects the total production of vitamin D from both endogenous and exogenous sources such as exposure to UVB radiation and intake of vitamin D from different dietary forms (Wang, 2008).

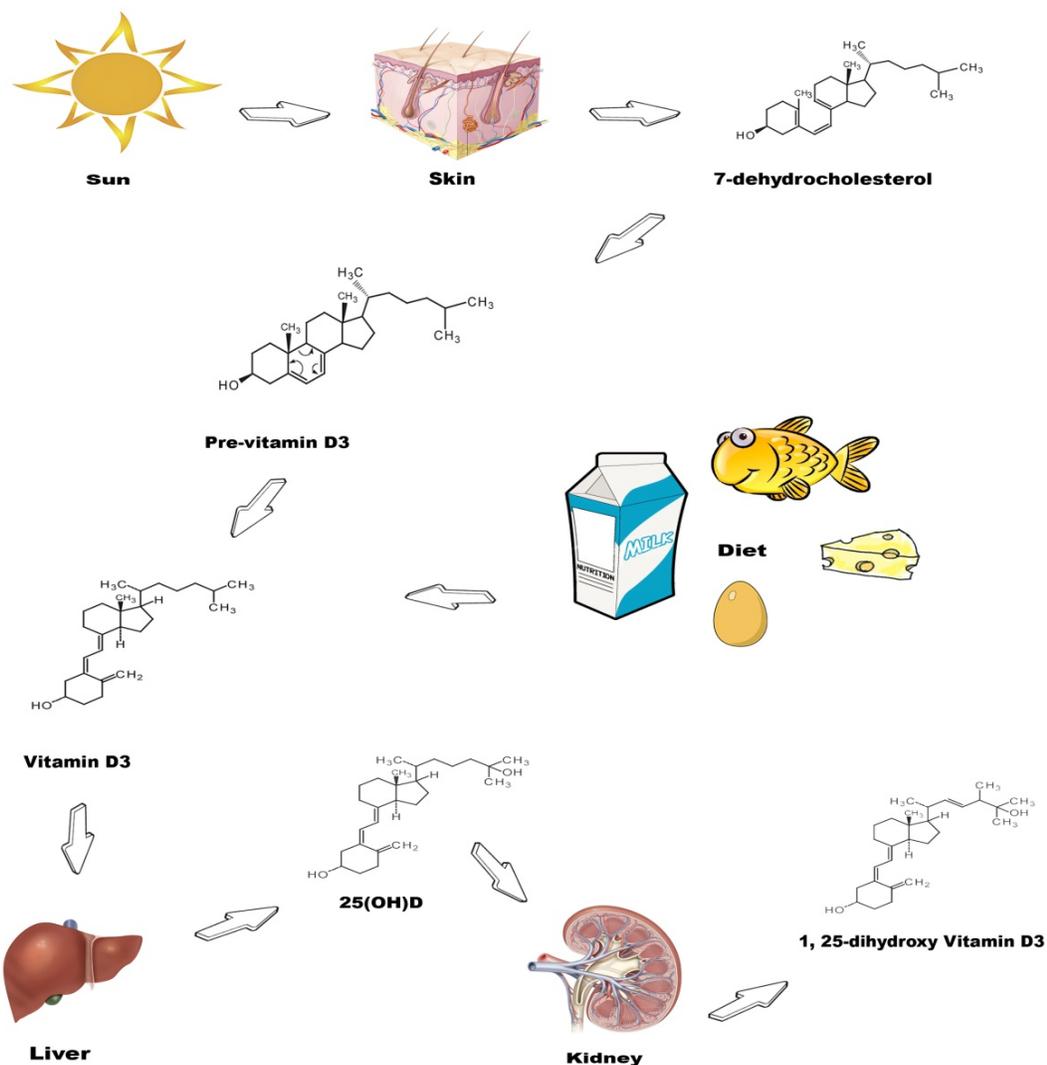


Figure 1. Vitamin D3 synthesis, activation, and catabolism.

Metabolism of vitamin D. Synthesis of vitamin D3 occurs in the skin where 7-dehydrocholesterol is converted to pre-vitamin D in response to UVB exposure. Vitamin D3 is obtained from pre-vitamin D3 in the skin or by intestinal absorption of dietary components and binds to vitamin D-binding protein in the circulation where it is transported to the liver. Vitamin D3 is then hydroxylated by liver 25-hydroxylase (CYP2R1) into 25(OH)D₃. 25(OH)D₃ is then hydroxylated in the kidney by 1- α -hydroxylase (CYP27B1), generating the active hormone form 1,25(OH)₂D.

Vitamin D function is best known for its role in the regulation of calcium homeostasis in the body. Additionally, through the reduction of urinary calcium loss and promoting calcium absorption from food in the gut, vitamin D exerts important functions in skeletal development and normal mineralization of bone (Holick, 2003). Without sufficient vitamin D bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children (Mughal, 2011) and osteomalacia in adults (Lips, 2011; Nagata, 2011). It also plays a vital role in the prevention of osteoporosis in adults. More recently, literature has indicated that vitamin D not only plays a role in calcium homeostasis but other important metabolic actions such as its role in cell proliferation and the immune system (Christakos et al., 2007), involvement in the inhibition of carcinogenesis (Drake & Ng, 2010) and an important role in reduction in the onset of other inflammatory diseases such cardiovascular diseases and T2D (Anagnostis, Athyros, Adamidou, Florentin, & Karagiannis, 2010; Roth, 2007).

Recently, vitamin D status has been found to play an important role on β -cell function, insulin resistance, insulin sensitivity and hence the development of T2D. Although vitamin D function is best known for calcium homeostasis it is believed to play a role in β -cell function, which may be mediated by the binding of circulating 1, 25-dihydroxyvitamin D to the vitamin D receptor in the β -cells. Alternatively, vitamin D could function through activation of 25(OH)D by 1-alpha-hydroxylase, which has been shown to be expressed in β -cells (Peechakara & Pittas, 2008). Vitamin D may also exert its effects by regulation of the extracellular calcium levels and calcium influx through the β -cells, therefore promoting insulin secretion, which is a calcium-dependent process

(Sheshadri, 2011). Any changes in calcium flux may negatively effects β -cell secretory function (Palomer, Gonzalez, Blanco-Vaca, & Mauricio, 2008; Pittas et al., 2006).

Mechanism of vitamin D action

Mechanism of action of vitamin D in the active form $1,25(\text{OH})_2\text{D}$ is mediated by binding to the Vitamin D receptor (VDR), where $1,25(\text{OH})_2\text{D}$ can then exert its actions on target tissue (Palomer et al., 2008). VDR functions as a ligand-dependent transcription factor for many genes that can bind to a number of vitamin D metabolites. VDR belongs to the super family of nuclear receptors that include steroid, thyroid and retinoic acid receptors (Baker et al., 1988). Before VDR can exert its effects on the cell it must first form a heterodimer with retinoid X receptor (RXR) (Hibler, 2010) (Figure 2). These dimers may inhibit or stimulate transcription of target genes through their binding to co-regulators, which may be stimulatory (co-activators) or inhibitory (co-suppressors). Different tissues in the body may have different amount of co-regulators, which indicates to some extent, tissue specificity for the actions of $1,25(\text{OH})_2\text{D}$ (Bikle, 2010).

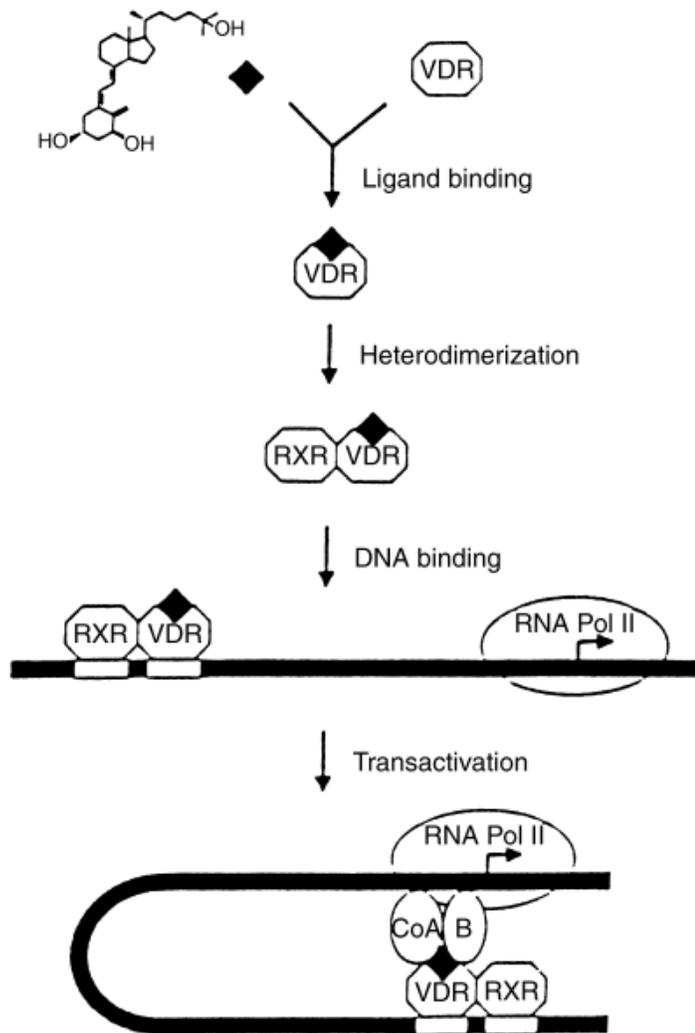


Figure 2. Transcriptional control of gene expression by 1,25(OH)₂D₃. The diagram shows the key steps involved in transcriptional regulation of 1,25(OH)₂D₃: (1) Ligand binding to the vitamin D receptor (VDR); (2) heterodimerization of VDR with retinoid X receptor (RXR); (3) binding of the VDR/RXR complex to the vitamin D response element; and (4) recruitment of components of the RNA polymerase II (Pol II) complex, including direct interactions with captivators (CoA) and transcriptional factor IIB (B). Modified from Brown, A.J. (1999). Therapeutically active vitamin D analogs. *Nephrology Exchange*, 8, 14–19.

Vitamin D, insulin secretion, sensitivity and resistance

Studies have shown that administration of vitamin D in both humans (Gedik & Akahn, 1986) and vitamin D deficient animals (Cade & Norman, 1986) improves insulin release and glucose tolerance (Mitri, Dawson-Hughes, Hu, & Pittas, 2011; Teegarden & Donkin, 2009). A review of the empirical literature that has attempted to evaluate the role of vitamin D in insulin secretion, sensitivity and resistance demonstrates some positive associations (Soares, Ping-Delfos, & She, 2008). For instance, Kayaniyil et al. (2010) measured levels of vitamin D, β - cell function and insulin resistance in 712 patients at-risk for the development of T2D. The research indicated that concentrations of vitamin D were associated with both β - cell function and insulin resistance. Based on these findings, Kayaniyil et al. (2010) argue that vitamin D may play some role in the development of T2D.

Nagpal, Pande and Bhartia (2009) also looked at the relationship between vitamin D and insulin sensitivity. The authors studied middle-aged males with central obesity that had not developed diabetes. Subjects were randomized to a vitamin D supplement group (provided once every two weeks) or a placebo group. Hepatic fasting insulin sensitivity, postprandial insulin sensitivity, lipid profile and blood pressure were measured at baseline and at the conclusion of the study (six weeks). The results of the investigation indicated that vitamin D improved postprandial insulin sensitivity in non-obese males that had not developed diabetes.

Mitri et al. (2011) have also considered the role of vitamin D on insulin sensitivity and β - cell function in individuals at-risk for the development of diabetes. The research conducted employed a randomized, 2 x 2 factorial design with subjects receiving

cholecalciferol (2000 IU once daily) or calcium carbonate (400 mg twice daily) for a period of 16 weeks. Pancreatic β - cell function was measured following an intravenous glucose tolerance test. Patients receiving calcium plus vitamin D did not have improved outcomes when compared with those receiving vitamin D alone. Overall, subjects had improved β - cell function; however no statistically significant improvements in A1c (glycated hemoglobin) attenuation were noted. Finally, Chiu et al. (2004) found that with the increase in 25(OH)D intake, insulin sensitivity was also increased. In addition, with low levels of vitamin D, β - cell function had decreased.

There is ample evidence indicating that vitamin D can impact insulin secretion, sensitivity and response. Some data also exist which provide further insight into possible associations between vitamin D and insulin response. Al-Sultan, Amin, Abou-Seif, and Al Naboli (2011) examined insulin sensitivity and β - cell function and their relation to vitamin D levels in obese and non-obese Saudis. While the results indicated that there were independent relationships between β - cell function and insulin sensitivity between the groups, when confounding variables such as smoking or physical activity were taken into consideration there were no differences in insulin sensitivity and β - cell function and their relationship to vitamin D levels between obese and non- obese Saudi men. Further, Alvarez, et al. (2010) found that vitamin D intake is associated with improved insulin sensitivity in African American, but not European women.

In Arab Americans 25(OH)D levels were assessed with regards to insulin resistance, metabolic syndrome, and glucose intolerance. Findings indicated that vitamin D insufficiency and deficiency is prevalent amongst this ethnic minority and lower

25(OH)D in men was associated with insulin resistance, components of the metabolic syndrome, and glucose intolerance. Lower 25(OH)D in women was associated with CVD risk factors (Pinelli, Jaber, Brown, & Herman, 2010). In 5787 Korean adults low 25(OH)D levels were associated with a high risk of T2D and findings were inversely associated with insulin resistance when subjects were overweight or obese (Choi, 2011). In contrast, Del Gobbo, Song, Dannenbaum, Dewailly, & Egeland (2011) found that serum 25(OH)D was not related to β -cell function or insulin resistance in 510 aboriginal Canadians with high risk of developing T2D. Given the scope of this data, further research is necessary to further understand the association between vitamin D and insulin action, in addition, ethnicity may play a critical role in the varied outcomes.

Vitamin D and type 2 diabetes

Longitudinal/Cohort Studies

Efforts to evaluate the role of vitamin D in the development of T2D have focused on broader epidemiological evaluations (e.g., longitudinal and cohort studies) to determine if vitamin D plays some causal role in the development of the disease (Jorde, Sneve, Hutchinson, Emaus, Figenschau, & Grimes, 2010). Grimnes et al. (2010) found after a 10-year follow up trial of those without T2D there was an inverse association between 25(OH)D and development of T2D. This association however was lost after controlling for body mass index.

Kirii, et al. (2009) utilized a cohort study of healthy subjects in Japan that did not have diabetes. These subjects were evaluated five years after initiation of the study to assess the number of individuals that had developed diabetes and their vitamin D intake.

The results indicated that there was no correlation between vitamin D intake and the development of T2D. Pittas, et al. (2006) through the Nurses Health Study followed 83,779 women who had no history of diabetes but had cardiovascular disease or cancer. Follow-up data assessed the development of T2D at 2-4 years of the study. Dietary vitamin D intake was not statistically significant, and there was no correlation between developing T2D and vitamin D intake. However, when participants consumed vitamin D combined with calcium, which was assessed through a food frequency questionnaire, there was a 33% reduction in risk of T2D.

Two cohort Finnish groups were studied by Laaksonen et al. (2010) between 1978-1980 and 2000-2001. They comprehensively evaluated lifestyle factors and their implications for the development of T2D. In this study, five modifiable lifestyle factors were used to define low risk lifestyle level: BMI less than 25, occasional or regular exercise (approximately 30 minutes or more per day), no smoking, limited alcohol consumption (1–99 g/week in women and 1–199 g/week in men), and vitamin D consumption above the median, with serum vitamin D levels above 39 nmol/L in the first cohort (1978-1980) and above 44 nmol/L in the second cohort (2000-2001). Analysis of cohort data over a 10-year follow-up period indicated that 82 percent of T2D cases developed during the study could be attributed to poor lifestyle factors including vitamin D deficiency. Even though the authors were not able to identify the role of vitamin D and its role in the development of T2D, the research indicated that Vitamin D is vital to a healthy lifestyle and the prevention of T2D.

Randomized controlled/Cross-sectional studies

Studies undertaken to evaluate vitamin D and its role in T2D have focused on cross sectional and randomized control studies to evaluate the impact of vitamin D supplementation for patients with diabetes (Jorde & Figenschau, 2009) and their prevalence. For instance, Witham, Dove, Dryburgh, Sugden, Morris and Struthers (2010) examined the use of different vitamin D doses on vascular markers for patients with T2D. The results of the investigation demonstrate that while reductions in systolic blood pressure and natriuretic peptide levels were noted, no statistically significant improvements in insulin resistance or A1c were found.

Sabherwal, Bravis, & Devendra (2010) reported conflicting data who performed a similar study using South Asian patients with T2D. In this research, the authors found that vitamin D supplementation decreased both A1c levels and weight. Data collected for this investigation was obtained from retrospective chart analysis of patients that had and had not received vitamin D and calcium supplementation. Sabherwal et al. (2010) found that while vitamin D was important in reducing A1c and weight, the moderating role of calcium in these processes must also be reviewed. Based on the available data, the authors were not able to separate out the specific role of vitamin D in reducing A1c.

Research undertaken by Sugden, Davies, Witham, Morris and Struthers (2008) further demonstrated the role that vitamin D may play in the progression of T2D. Specifically, these authors evaluated the use of a single high dose of vitamin D to improve endothelial function in patients with T2D in a double-blind randomized trial. The results of the investigation demonstrated that high doses of vitamin D intake during the winter months may improve endothelial function in patients with T2D. Improving

endothelial function is viewed as essential to preventing the development of cardiovascular disease associated with T2D. Scragg, Sowers and Bell (2004) performed data analysis from the Third National Health and Nutrition Examination Survey (NHANES) with data collected between 1998-1994. They found an inverse association between 25(OH)D and diabetes (associated with insulin resistance) in Mexican Americans and Whites adjusting for sex, age, BMI, leisure activity and quartiles of the year. These results were not found in Blacks.

Vitamin D and sun exposure in the Sunshine State

Melanin and sun exposure play a role in vitamin D formation and persons with diabetes are at a significant risk for vitamin D insufficiency. African Americans, Haitian Americans and Cuban Americans in the state of Florida may all have somewhat equal sun exposure in Miami throughout the year, with little seasonal variation. However, each ethnic group has different skin color: African Americans having the greatest amount of melanin compared to the majority of Cuban Americans who have the least amount of melanin and the fairest skin tone. African Americans are particularly at risk for vitamin D insufficiency because their darker skin color limits the amount of ultraviolet light that penetrates, thereby reducing cutaneous synthesis of vitamin D₃ (Aloia et al., 2008). In fact melanin skin pigmentation is an effective natural sunscreen. Skin pigmentation can reduce the UVB from penetrating the skin by as much as 99% which has the same effect as applying sunscreen of factor 15 (Holick, 2006). It has been found that vitamin D₃ levels in Blacks are less than in Whites when exposed to usual levels of sun exposure; also, Blacks have been found to have lower serum 25(OH)D concentrations in winter and

summer compared to their White counterparts (Harris & Dawson-Hughes, 1998; Harris, Soteriades, Coolidge, Mudgal, & Dawson Hughes, 2000; Scragg, Sowers, & Bell, 2004). Weaver and Fleet (2004) estimated that Blacks need 46–62 µg/d of vitamin D₃ supplementation. However, this assumption was made by Heaney, Davies, Chen, Holick and Barger-Lux (2003) and was based on a single study performed in white adults. Evidence and studies are currently lacking to make ethnically - specific recommendations for the required level of sun exposure or vitamin D intake at this time.

Vitamin D deficiency in the elderly

Vitamin D is obtained either from the diet, synthesis in the skin by the action of sunlight or in a supplemental form. Older people tend to have reduced endogenous production of the vitamin for a variety of reasons; hence they become more dependent on dietary sources to maintain adequate vitamin D status (DOH, 1998). However, vitamin D from the sun and even dietary sources occurs only sporadically and irregular intake of vitamin D regardless of its source can lead to chronic vitamin D inadequacy (Holick, 2006). Vitamin D deficiency, can particularly affect older adults since as people age, the skin cannot synthesize vitamin D as efficiently and the kidneys are less able to convert vitamin D to its active hormone form (Need, Morris, Horowitz, & Nordin, 1993). Decreased vitamin D in the elderly may be due to poor diet (Russell & Suter, 1993), being homebound or living in nursing homes (Webb, Kline, & Holick, 1990). In addition, the darker skinned elderly such as African Americans and Haitian Americans may be at even greater risk of vitamin D deficiency due to their increased skin pigment because ultraviolet rays-mediated synthesis of vitamin D is already greatly reduced (Clemens,

Adams, Henderson, & Holick, 1982). In addition, it has been found that up to 80 percent of Blacks and Hispanics have lactase deficiency so do not consume milk and cheese which have commonly fortified vitamin D (Swagerty, Walling, & Klein, 2002; Webb et al., 1990). Other reasons for lack of vitamin D from sun exposure are as follows: living in northern latitudes (for example New England), women who wear modest attire and head coverings for religious reasons, and people with occupations that prevent sun exposure (IOM, 1997; Webb, Kline, & Holick, 1988).

Vitamin D receptor

The Vitamin D Receptor (VDR) is expressed in many cells, including β - cells of the pancreas and adipocytes; and, may therefore play a role in glucose homeostasis, insulin resistance and insulin secretion (Oh & Barrett-Connor, 2002; Tworowska-Bardzinska, Lwow, & Kubicka, 2008). Vitamin D in the form of $1,25(\text{OH})_2\text{D}$ is mediated by VDR (Teegarden et al., 2009). Allelic differences in the VDR gene may also contribute to the genetic predisposition to certain diseases. As vitamin D modulates insulin secretion, it is feasible that genetic variants of the VDR gene may contribute to the development of T2D (Bid, Konwar, & Aggarwal, 2009).

VDR polymorphisms and insulin secretion

Vitamin D and its receptor complex may play a role in β -cell insulin secretion. There are three studies that looked at the association between VDR polymorphisms and insulin secretion. Hitman, Mannan, McDermott, Aganna, Ogunkolade, Hales and Boucher (1998) studied insulin secretion and its association with VDR polymorphism in

171 vitamin D deficient Bangladeshi Asian participants. Their findings indicated that there was an association between the VDR gene polymorphisms and insulin secretion particularly in the (AA) genotype. In contrast a later study conducted by Ogunkolade et al. (2002) found in 143 healthy Bangladeshis *TaqI* genotype independently predicted insulin secretion index, with (tt) genotypes showing the greatest insulin secretion index. One last study looked at VDR polymorphism of *BsmI* and its association with T2D and android type obesity. The study subjects consisted of 49 Caucasians with T2D, 29 with obesity but without T2D and 138 healthy subjects. It was found that highest postprandial C-peptide was in (BB) genotypes (wildtype homozygous genotype) that had T2D and obesity; thus, the VDR gene might play a role in the pathogenesis of T2D through the influence of β -cells secretory capacity (Speer et al., 2001). Although some studies have found a positive association with VDR polymorphisms and insulin secretion, there is a lack of studies indicating a consistent association.

VDR polymorphisms and insulin resistance

Low levels of Vitamin D may cause insulin resistance in the general population. It may also be one of the pathogenic mechanisms for abnormal glucose/insulin metabolism (Rammos, Tseke, & Ziakka, 2008). Few of the studies have looked at the association between insulin resistance and VDR polymorphisms, which may contribute to low levels of vitamin D regardless of diet, sun exposure and supplementation (Rammos et al., 2008).

One study conducted with 49 healthy glucose tolerant Caucasians, assessed fasting insulin and glucose levels (Chiu, Chuang, & Yoon, 2001) found that *FokI* polymorphism at the VDR gene was associated with insulin sensitivity. The ff/Ff

genotypes had higher fasting insulin and HOMA- IR (insulin resistance) than (FF). Also the *FokI* genotype independently predicted HOMA-IR (but not beta cell function).

The *BsmI* polymorphism frequency was assessed in 351 randomly selected Polish postmenopausal women where they found that *BsmI* is not associated with obesity and/or insulin resistance (Tworowska-Bardzinska, Lwow, & Kubicka, 2008). No difference was found in fasting insulin, glucose or fasting insulin resistance index between the genotypes (Alvarez & Ashraf, 2010), hence *BsmI* did not appear to be a predisposing factor for insulin resistance or obesity. Another study with the male Polish population investigated *FokI* and *BsmI* polymorphisms (Filus et al., 2008). Men ($n = 176$) were randomly selected and were between the ages of 25-65 years and with a mean BMI of 28.06 kg/m². It was found that the VDR polymorphism *BsmI* (BB) carriers seemed to have a higher BMI, whereas the *FokI* VDR polymorphism appeared to affect insulin sensitivity where the FF/ff genotype was associated with greater fasting insulin than (ff). *BsmI* was also measured in 1539 healthy male German soldiers with regard to their physical activity level (low and high) (Ortlepp, Metrikat, & Albercht, 2003). VDR genotype (BB) was associated with altered fasting blood glucose levels only in subjects with low physical activity.

The Rancho Bernardo (2002) cross-sectional study of 1,545 older Caucasian adults found that although the frequencies of *ApaI*, *BsmI* and *TaqI* polymorphisms did not differ between persons with or without diabetes, the frequency of the (aa) genotype of *ApaI* polymorphisms was marginally higher in persons with T2D ($p = 0.06$) and fasting glucose was also higher in (aa) genotype of *ApaI* (Oh & Barrett-Connor, 2002). In contrast HOMA-IR was higher in (BB) genotype *BsmI* (Alvarez & Ashraf, 2010). The

authors indicate that *Apal* polymorphism may be associated with glucose tolerance independent of defective insulin secretion; and, that *BsmI* polymorphism may be associated with insulin resistance (Oh & Barrett-Conor, p. 356).

Results from studies of insulin resistance, secretion and VDR polymorphisms, have been inconclusive and contradictory. Therefore further studies are needed to explore the relationship between VDR polymorphisms in genetically diverse populations.

VDR polymorphisms and type 2 diabetes

Although VDR gene is a candidate to study polymorphisms and its association with T2D, there are to date a few studies on the topic. Dilmeç, Uzer, Akkafa, Kose and Van Kuilenburg (2010) aimed to investigate whether there was an association between the VDR gene and polymorphisms of *Apal* and *TaqI* in a Turkish population with T2D. The research involved 241 participants, 72 with T2D and 169 healthy controls. Although the VDR genotype in patients with T2D was higher (19.4%) than those without T2D (11.2%) there were no significant differences. No evidence to support the association between *Apal* and *TaqI* with T2D were found (Dilmeç, Uzer, Akkafa, Kose, & Van Kuilenburg, 2010).

Another study conducted in 1998 also looked at the *Apal* and *TaqI* in 89 Guadeloupe Indians with T2D and 100 controls; and they also found no statistical differences in genotype frequencies between cases and controls (Boullu-Sanchis, Lepretre, & Hedelin, 1999). However, a significant association of age ($p = 0.002$) and waist-hip ratio ($p = 0.013$) with *TaqI* genotypes of VDR gene polymorphism was present. Similar results were found by Malecki, Frey, and Moczulski (2003) with Polish adults.

Adults ($n = 308$) with T2D and controls ($n = 240$) were assessed for associations between T2D and *TaqI*, *BsmI*, *FokI* and *ApaI* and found that there was no association between the four examined VDR polymorphisms with T2D in the Polish population.

Ye, Reis, and Dubois-Laforgue (2001) examined *BsmI*, *TruI*, *ApaI* and *TaqI* in 309 French subjects with T2D and in 143 controls; although no association was reported between the VDR gene and T2D the authors found VDR gene polymorphisms were associated with the susceptibility to obesity (BMI) in subjects with early-onset T2D. Finally, Vélayoudom-Céphise et al. (2011) in a cross-sectional study looked at 277 Caribbean patients with T2D and assessed their vitamin D levels, VDR polymorphisms *FokI*, *BsmI*, *ApaI* and *TaqI* and cardiovascular risk factors. Findings indicated that *FokI* and *ApaI* polymorphism were associated with vitamin D deficiency and those participants carrying the (aa) genotype of the *ApaI* Single Nucleotide Polymorphism (SNP) or (f) of *FokI* SNP may be protective of vitamin D deficiency.

Although the VDR gene is a good candidate for susceptibility to several diseases such as T2D (Dilmec et al., 2010), there has been little published data on VDR polymorphisms in the darker skinned populations (Vélayoudom-Céphise et al., 2011) or in Cuban Americans, Haitian Americans, and African Americans.

In summary, there has been few published data on vitamin D, VDR polymorphisms and insulin resistance, insulin sensitivity and β -cell function in the darker skinned populations including Haitian Americans, and African Americans. Hispanic minorities such as Cuban Americans also have limited research where most studies focus on the Caucasian populations. It is therefore important to add to the growing body of knowledge on the role vitamin D, VDR polymorphisms and pancreatic function in the

progression of T2D in these ethnic groups, since evidence is still inconclusive and lacking. The results of this study will generate data to expand our existing knowledge of the role ethnicity plays in chronic diseases. It is anticipated that further exploratory studies will be needed before intervention studies can be designed and implemented.

III. SERUM VITAMIN D INSUFFICIENCY AND DIABETES STATUS IN THREE ETHNIC GROUPS

Abstract

Aim: To determine whether there is a relationship between 25-hydroxyvitamin D (25(OH)D) levels and ethnicity and whether the relationship varies by diabetes status.

Methods: This cross sectional study included Haitian Americans ($n = 253$), Cuban Americans ($n = 199$), and African Americans ($n = 248$), with and without type 2 diabetes (T2D), from Florida, U.S. Participants were recruited through mailing lists (Cuban Americans and African Americans) and community-based sources (Haitian Americans). Information was obtained on participants' socio-demographic data, smoking history, and medication(s) used. Height and weight were measured using a SECA balance scale (Seca Corp., Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m^2 . Dietary intake was measured using the semi-quantitative food frequency questionnaire. Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10).

Results: Adjusted logistic regression analysis indicated that having T2D was associated with lower 25(OH)D levels as compared to participants without T2D regardless of ethnicity, age, gender, BMI, vitamin D intake, total calories intake, smoking, seasonal pattern ($p < 0.001$). Participants with T2D were 2.3 times more likely to have vitamin D insufficiency compared to participants without T2D, 95% CI (1.56 to 3.44).

Adjusted logistic regression analysis using Holms modified Bonferroni to correct for multiple comparisons indicated that there were significant differences in the odds of having insufficient 25(OH)D levels by ethnicity ($p < 0.001$), diabetes status ($p < 0.001$), and their interaction ($p < 0.001$). The covariates included in the model were age, gender, BMI, vitamin D intake, total calorie intake, smoking and seasons. Only Cuban Americans without T2D were significantly lower in the odds of having insufficient 25(OH)D from all other groups.

Conclusion: Of the three ethnicities studied, Cuban Americans had the highest vitamin D levels regardless of diabetes status. Future studies should assess skin color, sun exposure and vitamin D receptor polymorphism and other factors that may have influenced the higher vitamin D levels in this ethnic group. Additionally, clinical studies may be warranted in ethnic groups to determine whether safe optimum doses of vitamin D could correct insufficiency and play a role in the prevention of T2D.

Introduction

Low 25-hydroxyvitamin D (25(OH)D) levels have become an epidemic that only recently has been recognized in many populations around the world (Grant & Holick, 2005). Blacks are particularly vulnerable to vitamin D insufficiency primarily due to their darker skin color, which limits the amount of ultraviolet light (UVB) that penetrates, in turn, reducing cutaneous synthesis of vitamin D₃ (Forrest & Stuhldreher, 2011). Black Haitian Americans and African Americans are usually placed in the same ethnic category in most studies and in data banks such as the National Health and Nutrition Examination Survey (NHANES) (Dawson-Hughes, 2004). As a result, few studies have been conducted on ethnic groups such as Haitian Americans as a subgroup of Blacks.

Attention to minority groups is essential, in order to identify different characteristics distinctive perhaps to their ethnicity. For example, Haitian Americans have been found to have different diets and ethnic characteristics as compared to African Americans (CDC, 2011; De'silets, Rivard, Shatenstein, & Delisle, 2007; Scott, McDougale, Schwirian, & Taylor, 2010). Hispanics also exhibit a high prevalence of vitamin D deficiency (Forrest & Stuhldreher, 2011). Cuban Americans represent an important subgroup of Hispanics, with a total of 1.8 million residing in the United States in 2010, an increase of 44% since year 2000 (Ennis, Rios-Vargas, & Albert, 2011). Although the prevalence of diabetes among Cuban Americans (9%) is slightly lower than that of Puerto Ricans (11%) and Mexican Americans (10%) (Pabon-Nau, Cohen, Meigs, & Grant, 2010), the prevalence is still 1.3 to 1.5 times higher than among Whites (Stern & Mitchell, 1995). Cuban Americans represent the third largest Hispanic subgroup; however, there is limited research pertaining to vitamin D status or diabetes in this group

(Ennis, Rios-Vargas, & Albert, 2011). Hispanics and Blacks also have a higher risk of T2D as compared to Whites (CDC, 2011). The relationship between T2D and vitamin D has been associated with the function of the β -cells of the pancreas, where vitamin D levels have been found to play an important role in insulin resistance, insulin sensitivity, and, possibly, the development of T2D (Baynes, Boucher, Feskens, & Kromhout, 1997; Gerich, 2003). Therefore, the objective of this study was to investigate the association between 25-hydroxyvitamin D (25(OH)D) levels and diabetes status in three ethnic groups: Cuban Americans, Haitian Americans, and African Americans.

Methods

Participants

This cross-sectional study analyzed data from three ethnic minority groups with ($n = 373$) and without ($n = 327$) T2D, aged 35 years and older.

Data Collection

Participants were initially recruited by random selection from randomly generated mailing lists. The lists of addresses were purchased from Knowledge Base Marketing, Inc., Richardson, TX, U.S. Ten thousand letters, in English and Spanish along with an invitation flyer in both English and Spanish, were mailed to Cuban Americans. Of the participants who received letters, 4% ($n = 388$) responded. Additionally, approximately 7,550 letters were mailed to African Americans with and without T2D. Of the participants who received letters, again 4% ($n = 256$) responded. Because we did not have a similar mailing list database for Haitian Americans, we recruited these participants

from community-based sources ($n = 259$): (a) local diabetes educators and community health practitioners in Miami-Dade and Broward Counties; (b) Florida International University faculty, staff, and students; (c) several active adult apartment complexes; and (d) print advertisements and radio advertisements. When the calculated sample size for each ethnic group yielded sufficient power to detect significance differences between major variables, including vitamin D, diabetes status, and ethnicity, the recruitment of participants was stopped.

Interested participants were interviewed by phone, at which time the study purpose was explained and age, self-identified ethnicity (Cuban American, Haitian American, and African American), gender, self-reported T2D status (confirmed by fasting plasma glucose (> 126 mg/dL) and/or A1c ($<6.5\%$), years since diagnosis, and initial treatment modalities (oral glucose-lowering drugs and insulin) were confirmed. Participants who did not qualify for the study ($n = 28$) were younger than 35 years old ($n = 12$), of another ethnicity ($n = 5$), or had a chronic disease or illness such as cancer, HIV infection, kidney failure, or hepatitis ($n = 11$), as these conditions could influence the main outcome of the study. This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10). Written consent in English, Spanish, or Creole was obtained from the participants on their first visit to the laboratory. A total of 19 participants (Cuban Americans = 7; Haitian Americans = 8; and African Americans = 4) who reported not having diabetes were reclassified in the study as having T2D according to American Diabetes Association (ADA) standards. These participants were provided with a copy of their laboratory results and referred to their physicians. Participants with insufficient amounts of blood serum or an out-of-range coefficient of

variance were excluded ($n = 160$). A total of 700 participants (Cuban Americans = 199; Haitian Americans = 253; and African Americans = 248) were included in the final data analysis.

Measures

Participants were asked to complete standard self-administered questionnaires on site. For detailed questionnaires, trained interviewers were present to interview the participants and help them to complete the questionnaire. Data on participants' socio-demographic characteristics, smoking history, and medication(s) used were obtained. Height and weight were measured using a SECA balance scale with a stadiometer (Seca Corp, Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m^2 .

Assessment of dietary intake

Dietary intake was measured using the semi-quantitative food frequency questionnaire (FFQ; 97GP 2006 version copyrighted at Harvard University, Boston, MA, U.S.), developed by Walter C. Willett, which has been extensively validated and standardized in several multiethnic population-based studies (Satia-Abouta et al., 2003; Willett et al., 1985). Willett's FFQ also has been validated exclusively for the Cuban American population (Nath & Huffman, 2005). On the FFQ, participants self-report average consumption of specified amounts of various foods and vitamins over the past year. Daily servings for food groups were calculated by summing frequency factors for all related food items. Macro- and micro-nutrient intakes were calculated by multiplying

frequency of consumption by the nutrient value of the food item obtained from the Harvard University Food Composition Database. Each of the completed FFQs was reviewed for incomplete answers or blank responses before the interview was completed.

Blood collection

Twenty ml of venous blood was collected from each participant, after an overnight fast (at least 8 hours), by a certified phlebotomist using standard laboratory techniques. Blood samples were collected in two tubes: a Vacutainer Serum Separator Tube, (SST) for analysis of glucose and vitamin D, and another tube containing ethylenediamine tetra-acetic acid (EDTA) for the analysis of glycosylated hemoglobin (A1c). After complete coagulation (30-45 minutes), the SST sample was centrifuged at 2,500 RPM for 30 minutes. The serum was transferred from the spun SST into one labeled plastic tube for glucose and vitamin D analysis.

Glucose levels were measured by hexokinase enzymatic methods. A1c percentages were measured from whole blood samples using the Roche Tina Quant method by Laboratory Corporation of America (LabCorp®), Miami, FL, U.S. Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). The manufacturer's protocol was followed to perform this competitive assay, according to which the standards and samples were diluted with biotin-labeled 25(OH)D. For this study, the interassay coefficient of variation (CV) for ELISA of less than 10.3% was accepted (Bodnar et al., 2007). The cutoff point for insufficient serum 25(OH)D levels was < 75 nmol/L (IOM,

2011). Serum 25(OH)D was used because it is considered the most reliable measure of overall vitamin D status (Misra, Pacaud, Petyk, Collett-Solberg, & Kappy, 2008).

Data Analysis

All statistical analyses were performed using SPSS (Version 18.0, Chicago, IL, U.S.). Differences in mean values by ethnicity and diabetes status were assessed using the Student's *t*-test and analysis of variance (ANOVA), for numerical values, and χ^2 test for categorical variables. Logistic regression analysis was used to determine the relationship between the dependent variable, serum 25(OH)D levels (sufficient/ insufficient), and the independent variables, ethnicity (Haitian American, Cuban American, and African American), diabetes status (yes/no), and their interaction. The final model was adjusted for potential socio-demographic, lifestyle, and physical factors (age, gender, BMI, vitamin D intake, total calorie intake, smoking, and season). The post-hoc Holm's modified Bonferroni method was used to correct for multiple comparisons. Significance level for all analyses was set at $p < 0.05$.

Results

The study included 700 participants (Cuban Americans, Haitian Americans = 253, = 199, and African Americans = 248). Table 1 presents characteristics of study participants by ethnicity. Cuban Americans were significantly older and had a greater percentage of female participants than did Haitian and African Americans ($p < 0.05$). Cuban Americans also had significantly higher serum 25(OH)D levels compared to African Americans and Haitian Americans ($p < 0.05$). African Americans had a

significantly higher BMI, a higher proportion of smokers, and a higher caloric intake as compared to Cuban Americans and Haitian Americans ($p < 0.05$). Additionally, African Americans had a higher vitamin D intake than did Haitian Americans and Cuban Americans ($p < 0.05$).

Table 2 shows the characteristics of study participants by diabetes status. Participants with T2D were significantly older ($p < 0.001$), had a higher BMI ($p < 0.001$), had lower caloric intake ($p = 0.005$), and had lower serum 25(OH)D levels ($p < 0.001$) as compared to participants without T2D.

There were significant differences in age, BMI, ethnicity, diabetes status, and season by vitamin D status (Table 3). Participants in the insufficient 25(OH)D group were significantly younger ($p = 0.003$) and had a higher BMI ($p < 0.001$). This group also had a lower percentage of Cubans Americans ($p < 0.001$) and a higher percentage of individuals with T2D ($p < 0.001$). A greater percentage of those tested in the spring-summer season were sufficient (31.1%) than were those tested in the winter (18.5%) ($p < 0.001$).

Adjusted logistic regression analysis indicated that having T2D was associated with lower 25(OH)D levels as compared to participants without T2D regardless of ethnicity, age, gender, BMI, vitamin D intake, total calories intake, smoking, seasonal pattern ($p < 0.001$). Participants with T2D were 2.3 times more likely to have vitamin D insufficiency compared to participants without T2D, 95% CI (1.56 to 3.44)(not shown).

Table 4 shows the adjusted logistic regression analysis using the Holm's modified Bonferroni method to correct for multiple comparisons. The adjusted logistic regression indicated significant differences in the odds of having insufficient 25(OH)D levels by

ethnicity [χ^2 (df = 2, $N = 700$) = 80.0, $p < 0.001$], diabetes status [χ^2 (df = 1, $N = 700$) = 45.0, $p < 0.001$], and their interaction [χ^2 (df = 2, $N = 700$) = 37.8, $p < 0.001$]. This model included covariates (age, gender, BMI, vitamin D intake, total calorie intake, smoking and seasons) which were statistically significant [χ^2 (df = 12, $N = 700$) = 157, $p < 0.001$] and explained 29.6% of the variance of 25(OH)D levels (Nagelkerke pseudo R-square); only Cuban Americans without T2D had lower odds of being vitamin D insufficient than Cuban Americans with T2D and African Americans and Haitian Americans with and without T2D.

Discussion

Our study found significant differences in vitamin D levels across diabetes status and ethnic groups. Primarily, we found that participants with T2D had lower serum 25(OH)D levels ($p < 0.001$) compared to participants without T2D, which is in agreement with previous research (Baynes et al., 1997). The mechanism of action of vitamin D in diabetes is not completely understood in humans; however, it is thought that vitamin D deficiency impairs insulin synthesis and secretion in β - cells of the pancreas, which results in the development of T2D (Gerich, 2003).

Additionally, Cuban Americans without T2D had the highest vitamin D levels as compared to Haitian Americans and African Americans. These differences among ethnicities may be caused by a number of factors. First, lower vitamin D levels in participants with black skin color (African Americans, and Haitian Americans) may be directly associated with their skin color. Nesby-O'Dell et al. (2002) reported that 10 times as many African American women as white women were deficient in vitamin D (\leq

37.5 nmol/L). Due to their darker skin, Blacks require greater sunlight exposure, as melanin skin pigmentation is an effective natural sunscreen. Darker-pigmented individuals would require longer periods of time in the sun (Zittermann, 2003). Second, Blacks may have limited absorption, resistance, or decreased ability to synthesize vitamin D in the body (Dawson- Hughes, 2004). Third, vitamin D receptor (VDR) polymorphisms may create metabolic disturbances, variations in insulin secretion, and genetic susceptibility to T2D (Ogunkolade et al., 2002). Fourth, based on our study African Americans had the highest BMI. It has been reported that there is an inverse association between serum 25(OH)D and BMI (Arunabh, Pollack, Yeh, & Aloia, 2003), whereby, it is proposed, that vitamin D is sequestered in fat cells (Need, Morris, Horowitz, & Nordin, 1993) and, with increased fat in the body, circulating vitamin D levels decrease.

The Third National Health and Nutrition Examination Survey found ethnic variations in 25(OH)D levels, whereby Whites had the highest 25(OH)D concentrations, followed by Mexican Americans and then Blacks, who had the lowest levels (Scragg et al., 2004). These results are in accordance with our study, which found that African Americans and Haitian Americans were most likely to have vitamin D insufficiency, with Cuban Americans being the least likely to have vitamin D insufficiency. Although skin pigmentation was not measured in our cohort, it was observed that all of the Cuban American participants had white skin pigmentation, which may have allowed them greater UVB exposure as compared to the Haitian Americans and African American participants.

Interestingly, in our study, and contrary to the findings in regard to Cuban Americans and African Americans, we found that Haitian Americans without diabetes were at the greatest risk for 25(OH)D insufficiency, when compared to Cuban Americans without T2D. However, differences in 25(OH)D in Haitian Americans with and without diabetes were not significantly different.

We did not find any association between covariates and the presence of T2D or vitamin D status to explain the increased vitamin D insufficiency found in the Haitian Americans without T2D. Therefore, we hypothesize that our findings for Haitian Americans may be due to skin color or genetic factors. These results also may be specific to the cohort that we studied and, as such, the findings cannot be generalized to other Haitians living in the U.S.

Participants in the insufficient 25(OH)D group were younger as compared to the sufficient group. Generally, it has been found that vitamin D deficiency can particularly affect older adults; as the skin ages, it cannot synthesize vitamin D as efficiently, and the kidneys are less able to convert vitamin D to its active hormone form (Need et al., 1993). In addition, our study found that African Americans had the highest intake of vitamin D as compared to their counterparts. The higher vitamin D intake was not the result of dietary supplementation; thus, it may have been due to the increased caloric intake demonstrated in this African American cohort (~2569kcal/day). However, this finding is contradictory to the findings of other studies, which have found that African Americans normally have low intakes of vitamin D because commonly fortified foods that contain vitamin D, such as milk and cheese, contain lactose; and it has been reported that up to 80% of Blacks and Hispanics have lactase deficiency (Swagerty, Walling, & Bell, 2004).

Data collection during spring-summer months showed that fewer participants had insufficient 25(OH)D compared to sufficient. Our results are consistent with those of Scragg et al. (2004), who also found a seasonal pattern, with 25(OH)D levels being higher in the summer months. Higher 25(OH)D levels in spring-summer months may be an indicator that more people are exposed to sun during this time and, thus, are less likely to have 25(OH)D insufficiency.

From our total cohort we can conclude that having T2D was associated with lower 25(OH)D levels after adjustment for covariates. However, we did not control for sun exposure and other unknown factors that may have affected vitamin D levels. The possibility that skin color may play an important role with regards to vitamin D levels requires further exploration with direct measures of skin color. In addition, genetic factors, quality of diet and lifestyle factors may each have an individual or combined effect on specific ethnic groups. For example our study found that although both Haitian Americans, and African Americans are Black they still possess other factors that make them unique to one another. African Americans had the highest BMI, energy intake, percentage of smokers and vitamin D intake, whereas Haitian Americans had the lowest BMI, energy intake, percentage of smokers and vitamin D intake. We did not find statistically significant differences amongst Haitian Americans and African Americans but rather found that both groups were highly likely to have 25(OH)D insufficiency.

This study had some significant strengths. It is the first study that distinguishes two Black groups (Haitian Americans and African Americans) as separate and independent ethnicities (with regard to vitamin D and diabetes status) and, as such, yields important information on ethnic differences. It also looks at the relationship between

ethnicity, diabetes status, and serum vitamin D levels, which provides insight into how the variables interact with one another.

There are a number of potential limitations in this study. First, sun exposure and skin color were not measured. This information could have provided an additional understanding of the role that these factors may have on vitamin D status. Second, dietary intake was self-reported; it is possible that dietary intake may have been over- or underestimated by different ethnic groups. However, the FFQ used has been validated in the Cuban American population (Nath & Huffman, 2005) and in other multiethnic population-based prospective and cross-sectional studies (Satia-Abouta et al., 2003; Willet & Sampson, 1985). Third, parathyroid hormone levels were not assessed in this study, which may have provided a better understanding of the vitamin D regulation in the three ethnicities.

Our findings indicate that being African American or Haitian American increases the likelihood of vitamin D insufficiency. We postulate that ethnicity, diabetes status, and possibly skin color contributed to our findings; however the association is still unclear. Vitamin D status is an important parameter that needs to be assessed in individuals at high risk for vitamin D insufficiency. Clinical studies may be warranted to determine whether safe optimum doses of vitamin D could correct insufficiency and play a role in the prevention of T2D. Future studies also may benefit from an examination of acculturation factors such as time of immigration, length of stay, and living dynamics of Haitian Americans and Cuban Americans, which may affect dietary intake and risk of disease. Other genetic factors, such as vitamin D receptor polymorphisms, may affect

vitamin D status and may play a role in the progression of T2D. Because evidence is still inconclusive or lacking, further research is recommended.

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Table 1. Characteristics of study participants by ethnicity

Variables	Ethnicity			<i>p</i>
	Cuban Americans (<i>n</i> = 199)	Haitian Americans (<i>n</i> = 253)	African Americans (<i>n</i> = 248)	
Age (years)	62.8 ± 11.6 ^a	56.0 ± 10.7 ^b	52.7 ± 9.5 ^c	<0.001
Gender (M) (%)	36.7 ^b	47.4 ^a	49.2 ^a	0.019
BMI (kg/m ²)	31.4 ± 6.0 ^b	29.2 ± 5.2 ^c	33.5 ± 7.9 ^a	<0.001
Smoking (yes) (%)	13.1 ^b	6.3 ^c	37.9 ^a	<0.001
T2D status (yes) (%)	55.3	53.0	52.0	0.784
Energy intake (kcal/d)	2264.9 ± 795.6 ^b	1817.5 ± 1104.2 ^c	2569.1 ± 1834.1 ^a	<0.001
Vitamin D intake (IU/d)	449.0 ± 340.3 ^b	379.1 ± 356.8 ^c	537.4 ± 474.1 ^a	<0.001
Vitamin D intake w/o suppl. (IU/d)	277.6 ± 177.1	277.6 ± 265.4	318.5 ± 309.6	0.142
25(OH)D (nmol/L)	82.1 ± 34.8 ^a	56.3 ± 19.4 ^b	54.2 ± 23.8 ^b	<0.001
Vitamin D suppl. (yes) (%)	2.5	6.3	12.5	<0.001

Data are means ± SD unless otherwise indicated. Ethnic groups with different superscripts are significantly different from each other. Holm's modified Bonferroni method was used to correct for multiple comparisons. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D; w/o = without; suppl = supplements.

Table 2. Characteristics of study participants by diabetes status

Variables	Participants		<i>p</i>
	without T2D (<i>n</i> = 327)	with T2D (<i>n</i> = 373)	
Age (years)	54.6 ± 10.7	58.7 ± 11.5	<0.001
Gender (M) (%)	45.6	44.5	0.778
BMI (kg/m ²)	30.0 ± 5.6	32.6 ± 7.4	<0.001
Smoking (yes) (%)	20.2	18.8	0.636
Energy intake (kcal/d)	2368.6 ± 1450.7	2072.8 ± 1305.3	0.005
Vitamin D intake (IU/d)	474.5 ± 409.0	438.1 ± 397.9	0.229
Vitamin D intake w/o suppl. (IU/d)	297.2 ± 279.4	287.6 ± 245.6	0.630
25(OH)D (nmol/L)	70.1 ± 33.3	56.6 ± 22.2	<0.001
Vitamin D suppl. (yes) (%)	9.2	5.9	0.099

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05.

Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D; w/o = without; suppl = supplements.

Table 3. Characteristics of study participants by 25-hydroxyvitamin D status

Variables	25(OH)D status		<i>p</i>
	Insufficient (<i>n</i> = 522)	Sufficient (<i>n</i> = 178)	
Age (years)	56.0 ± 11.6	58.9 ± 10.1	0.003
Gender (M) (%)	44.3	47.2	0.496
BMI (kg/m ²)	31.9 ± 7.0	29.8 ± 5.4	<0.001
Ethnicity (%)			<0.001
Cuban American	19.5	54.5	
Haitian American	40.6	23.0	
African American	39.8	22.5	
T2D status (yes) (%)	58.2	38.8	<0.001
Smoking (yes) (%)	20.3	16.9	0.315
Energy intake (kcal/d)	2207.1 ± 1456.5	2222.3 ± 1139.5	0.899
Inadequate vitamin D intake (%)	57.1	51.7	0.210
Vitamin D suppl. (yes) (%)	7.7	6.7	0.686
Season (spring-summer) (%)	51.0	67.4	<0.001

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05.

Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; suppl = supplements; 25(OH)D = 25-hydroxyvitamin D. Serum 25-hydroxyvitamin D insufficiency <75 nmol/L; inadequate vitamin D intake (<400 IU/d).

Table 4. Odds of having insufficient 25-hydroxyvitamin D by ethnicity and diabetes status

Groups	OR	95% CI	<i>p</i>
CA without T2D	Reference ^a	-	-
CA with T2D	10.57 ^b	5.31 - 21.05	<0.001
AA without T2D	13.14 ^b	6.39 - 27.03	<0.001
HA with T2D	15.67 ^b	7.65 - 32.09	<0.001
AA with T2D	24.37 ^b	10.51 - 56.54	<0.001
HA without T2D	31.75 ^b	14.17 - 71.14	<0.001

^aReference category for *p* value. Groups with different superscripts are significantly different from each other, using the Holm's modified Bonferroni method to correct for multiple comparisons. Adjusted logistic regression analysis indicated significant differences in the odds of having insufficient 25(OH)D levels by ethnicity [χ^2 (df = 2, *N* = 700) = 80.0, *p* < 0.001], diabetes status [χ^2 (df = 1, *N* = 700) = 45.0, *p* < 0.001], and their interaction [χ^2 (df = 2, *N* = 700) = 37.8, *p* < 0.001]. The model included covariates (age, gender, BMI, vitamin D intake, total calorie intake, smoking and season) was statistically significant [χ^2 (df = 12, *N* = 700) = 157, *P* < 0.001] and explained 29.6% of the variance of 25(OH)D levels (Nagelkerke pseudo R-square). Abbreviations: T2D = Type 2 diabetes

IV. VITAMIN D RECEPTOR POLYMORPHISMS, VITAMIN D LEVELS, AND DIABETES STATUS IN THREE ETHNIC GROUPS

Abstract

Aim: To assess the relationship between serum 25(OH)D levels, vitamin D receptor (VDR) polymorphisms (*BsmI*, *TaqI* and *Apal*), and diabetes status in three ethnic groups (Cuban Americans, Haitian Americans, and African Americans) residing in South Florida.

Methods: This cross-sectional study included 749 participants (Cuban American = 267; Haitian American = 245; African American = 237) with and without type 2 diabetes (T2D) from Florida, U.S. Participants were recruited through mailing lists (African Americans and Cuban Americans) and community-based sources (Haitian Americans). Information was obtained on participants' sociodemographic data, smoking history, and medication(s) used. Height and weight were measured using a SECA balance scale (Seca Corp., Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m². Dietary intake was measured using a semi-quantitative food frequency questionnaire. Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). The single nucleotide polymorphisms (SNPs) *BsmI*, *TaqI*, and *Apal* were genotyped using PCR-RFLP and RT-PCR. All statistical analyses were performed using SPSS (Version 18.0, Chicago, IL, U.S.). For statistical analysis, Student's *t*-test, analysis of variance (ANOVA), χ^2 test, and logistic regression analysis were used. This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10).

Results: The study included 749 participants genotyped for *BsmI* and *TaqI*, and 734 participants for *Apal* polymorphism. *BsmI* polymorphism was not in Hardy-Weinberg equilibrium and was excluded from the study. *TaqI* and *Apal* genotype frequencies showed significant differences by ethnicity ($p < 0.001$), with Cuban Americans having a higher percentage of (tt) (12.4%), compared to African Americans (2.5%) and Haitian Americans (6.5%). After controlling for age, gender, and BMI, only Cuban Americans with T2D were 7.3 times more likely to be vitamin D insufficient 95% CI (1.97 to 26.9) ($p = 0.003$) if they had *TaqI* (TT) and (Tt). Participants with T2D had lower serum 25(OH)D ($p < 0.001$) levels than did those without T2D. There were no significant differences in combined genotype frequency of *TaqI* and *Apal* by diabetes status.

Conclusion: *TaqI* was found to be associated with vitamin D and ethnicity; it appears that the (tt) genotype in Cuban Americans may be protective against vitamin D insufficiency. In comparison, *Apal* was not associated with T2D or vitamin D levels in our cohort of Cuban Americans, Haitian Americans, or African Americans. Further work is needed to assess how the *TaqI* (tt) genotype is associated with higher vitamin D levels in Cuban Americans with T2D. Additionally, there is a need to explore how other potential VDR SNPs may influence or enhance plasma vitamin D levels.

Introduction

There has been a growing interest in the association between type 2 diabetes (T2D) risk and vitamin D levels. This association relates to the function of the β -cells of the pancreas, where low vitamin D levels are found to alter insulin resistance, secretion, and sensitivity and lead to impaired glucose metabolism (Baynes, Boucher, Feskens, & Kromhout, 1997; Chiu, Chu, Go, & Saad, 2004; Filus et al., 2008; Gerich, 2003). After Vitamin D is obtained from ultraviolet-B (UVB) sun exposure, food, and supplements, it is biologically inert and must undergo two hydroxylations in the body for activation. The first occurs in the liver, where vitamin D is converted into 25-hydroxyvitamin D (25(OH)D). The second hydroxylation occurs primarily in the kidneys, where it is enzymatically converted to the physiologically active 1,25-dihydroxyvitamin D (1,25(OH)₂D) (Mathieu, Gysemans, Bouillon, & Bouillon, 2005).

Serum 1,25(OH)₂D is the biologically active hormone form of vitamin D, and its mechanism of action is mediated by binding to the vitamin D receptor (VDR), where 1,25(OH)₂D can then exert its actions on target tissue (Palomer, Gonzalez, Blanco-Vaca, & Mauricio, 2008). VDR functions as a ligand-dependent transcription factor for many genes that can bind to a number of vitamin D metabolites. Because vitamin D deficiency impairs insulin synthesis and secretion of β -cells of the pancreas (Gerich, 2003), and the VDR is expressed in many cells, including the β -cells of the pancreas, VDR could play a direct role in glucose homeostasis, insulin resistance, and insulin secretion (Oh & Barrett-Connor, 2002). It is feasible that genetic variants of the VDR gene may contribute to the development of T2D (Bid et al., 2009), especially because it has been found that variants near genes involved in vitamin D transport affect vitamin D status (Wang, 2010). Few

studies have addressed the relationships among vitamin D levels, VDR polymorphisms, and diabetes. Vélayoudom-Céphise et al. (2011) indicated that, in participants with T2D, the single nucleotide polymorphisms (SNP) at *ApaI* and *FokI* restriction sites were associated with vitamin D deficiency, where the (aa) genotype of the *ApaI* and the (Ff or ff) genotypes of *FokI* SNP were found to be protective against vitamin D deficiency.

Different ethnicities may have variable levels of 25(OH)D, with a higher prevalence of vitamin D insufficiency in African Americans, Hispanics, and individuals with a high melanin concentration (Forrest & Stuhldreher, 2011). Low serum 25(OH)D in darker-skin individuals is attributed to the diminished synthesis of vitamin D due to high pigmentation of the skin (Bell, 1985).

The most commonly examined polymorphisms of the VDR gene are *BsmI* (rs1544410) *TaqI* (rs731236) and *ApaI* (rs7975232) (Seshadri & Tamilselvan, 2011), located on the 3' untranslated (UTR) region of the VDR gene. Although polymorphisms begin as mutations in a population, with time, they become true polymorphisms and may be associated with certain ethnicities. Thus, differences in the frequency of alleles among ethnic groups may result from the evolutionary processes and genetic behavior of the population (Uitterlinden, Fang, Van Meurs, Pols, & Van Leeuwen, 2004). So far, little is known about the relationship between ethnicity and VDR polymorphisms. Most studies have been in Caucasian populations. Blacks and Caribbeans have been studied to a lesser extent (Maleki, Frey, Moczulski, Klupa, Kozek, & Sieradzki, 2003; Uitterlinden et al., 2004; Vélayoudom-Céphise et al., 2011).

The aim of this study, therefore, was to assess the relationship between serum 25(OH)D levels, VDR polymorphisms (*BsmI*, *TaqI* and *ApaI*), and diabetes status in

three ethnic groups (Cuban Americans, Haitian Americans, and African Americans) who reside in South Florida. The three groups comprise a diverse population, representing differing ethnicities, skin pigmentations, and, likely, genetic predispositions.

Methods

Participants

In this cross-sectional study, data were from three ethnic minority groups, with ($n = 365$) and without ($n = 384$) T2D, aged 35 years and older.

Data Collection

Participants were initially recruited by random selection from randomly generated mailing lists, purchased from Knowledge Base Marketing, Inc., Richardson, TX, U.S. Ten thousand letters, along with an invitation flyer in both English and Spanish, were mailed to Cuban Americans. Of the participants who received letters, 4% ($n = 388$) responded. Additionally, approximately 7,550 letters were mailed to African Americans with and without T2D. Of the participants who received letters, again 4% ($n = 256$) responded. Because we did not have a similar mailing list database for Haitian Americans, we recruited these participants from community-based sources ($n = 259$): (a) local diabetes educators and community health practitioners in Miami-Dade and Broward Counties; (b) Florida International University faculty, staff, and students; (c) several active adult apartment complexes; and (d) print advertisements and radio advertisements. When the calculated sample size for each ethnic group yielded sufficient power to detect

significance differences between major variables, the recruitment of participants was discontinued.

Interested participants were interviewed by phone, at which time the study purpose was explained and age, self-identified ethnicity (Cuban American, Haitian American, and African American), gender, self-reported T2D status (confirmed by fasting plasma glucose (>126 mg/dL) and/or A1c ($<6.5\%$), years since diagnosis, and initial treatment modalities (oral glucose-lowering drugs and insulin) were confirmed. Participants who did not qualify for the study ($n = 28$) were younger than 35 years old ($n = 12$), of another ethnicity ($n = 5$), or had a chronic disease or illness such as cancer, HIV infection, kidney failure, or hepatitis ($n = 11$), as these conditions could influence the main outcome of the study. This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10). Written consent in English, Spanish, or Creole was obtained from the participants on their first visit to the laboratory. A total of 19 participants (Haitian Americans = 8; Cuban Americans = 7; and African Americans = 4) who reported not having diabetes were reclassified in the study as having T2D according to American Diabetes Association (ADA) standards. These participants were provided with a copy of their laboratory results and referred to their physicians. Participants with insufficient amounts of blood serum, out-of-range coefficient of variance, or missing or out-of-range VDR values were excluded. A total of 749 participants (Cuban American = 267; Haitian American = 245; African American = 237) were included in the final data analysis.

Measures

Participants were asked to complete standard self-administered questionnaires on site. For detailed questionnaires, trained interviewers were present to interview the participants and help them to complete the questionnaire. Data on participants' sociodemographic characteristics, smoking history, and medication(s) used were obtained. Height and weight were measured using a SECA balance scale with a stadiometer (Seca Corp., Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m².

Blood collection

Twenty ml of venous blood were collected from participants after an overnight fast (at least 8 hours) by a certified phlebotomist using standard laboratory techniques. Blood samples were collected in two tubes: a Vacutainer Serum Separator Tube (SST), for analysis of glucose and vitamin D, and another tube containing ethylenediamine tetraacetic acid (EDTA) for the analysis of glycosylated hemoglobin (A1c). After complete coagulation (30-45 minutes), the SST sample was centrifuged at 2,500 RPM for 30 minutes. The serum was transferred into a labeled plastic tube and stored at -70°C until used.

Determination of glucose and vitamin D levels

Glucose levels were measured by hexokinase enzymatic methods. A1c percentages were measured from whole blood samples with the Roche Tina Quant method by Laboratory Corporation of America (LabCorp, Miami, FL, U.S.). Serum

25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). The manufacturer's protocol was followed to perform this competitive assay, according to which the standards and samples were diluted with biotin-labeled 25(OH)D. For this study, the interassay coefficient of variation (CV) for ELISA was accepted at less than 10.3% (Bodnar et al., 2007). The cutoff point used to determine insufficient serum 25(OH)D levels was <75 nmol/L (IOM, 2011).

SNP genotyping

Blood samples were collected from each subject, and a proper amount of aliquot (400µl) was placed in 2.0ml cryo vials and stored at -70°C until DNA extraction. Total DNA (a mixture of genomic DNA and mitochondrial DNA) was extracted from the whole blood collected, according to the instructions of the user manual of the DNA extraction kit (QIAGEN, Valencia, CA, U.S.). The extracted DNA samples were stored at -20°C after measurements of DNA concentration.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping *BsmI* and *TaqI*, and RT-PCR was used for *ApaI*. PCR amplification was performed with PCR supermix high fidelity that contained 1.5 mM Mg²⁺ and two gene-specific primers. Primer sets purchased from Invitrogen/Life Technologies, Carlsbad CA, U.S., were as follows: for *BsmI* genotype, 5'CAACCAAGACTACAAGTACCGCGTCAGTGA-3' (sense) and 5'-AACCAGCGGAAGAGGTCAAGGG-3' (antisense) (NCBI, 2012); for genotypes of *TaqI*, 5'-GGTGGGATTGAGCAGTG-3' (sense) and 5'-ATGCTGCACTCAGGCTG-3'

(antisense), as described elsewhere (Oh & Barrett-Connor, 2002). The gene-specific primers for *BsmI* in this paper were designed based on GenBank nucleotide sequence databases with the following accession number: NG_008731, Homo sapien vitamin D (1,25- dihydroxyvitamin D3) receptor (VDR), RefSeqGene on chromosome 12. Forward: 58336- 58365. Reverse: 59136-59157 (NCBI, 2012).

PCR conditions for *BsmI* genotype were as follows: initial denaturation of 1 cycle for 3 min at 94°C; 39 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec; final elongation of 1 cycle at 72°C for 4 min. PCR conditions for *TaqI* genotype were as follows: initial denaturation of 1 cycle for 3 min at 94°C; 39 cycles at 94°C for 30 sec, 57°C for 30 sec, and 72°C for 60 sec; final elongation of 1 cycle at 72°C for 4 min.

The PCR products (15µl per sample) were digested for 5 hours at 65°C, with respective restriction enzymes *BsmI* (7.5 units) and *TaqI* (7.5 units) purchased from New England BioLabs (Ipswich, MA, U.S.). All products were separated by gel electrophoresis on 2% agarose gel in 1x TAE buffer and visualized by ethidium bromide staining under ultraviolet light.

DNA fragments of the SNP *Apal* were amplified from genomic DNA by TaqMan allelic discrimination assay. PCR amplification was as follows: Pre-PCR read of 1 cycle for 30 seconds at 60°C; holding stage of 1 cycle for 10 minutes at 95°C; 40 cycles for 15 seconds at 95°C and 1 minute at 60°C; post-PCR of 1 cycle at 60°C for 30 seconds and ending the reaction at 4°C holding. The SNPs *BsmI* and *TaqI* were amplified using the PCR- RFLP because there was not enough specificity provided by RT-PCR.

Data analysis

All statistical analyses were performed using SPSS (Version 18.0, Chicago, IL, U.S.). Differences in mean values among ethnicities and diabetes status were assessed using the Student's *t*-test and analysis of variance (ANOVA) for numerical values and χ^2 test for categorical variables. Stratification by ethnicity was necessary for data analysis, as VDR act differently in varied population groups and ethnicities (Uitterlinden et al., 2004). Logistic regression analysis was used to determine the relationship of serum 25(OH)D levels (sufficient/ insufficient), diabetes status (yes/no), SNP polymorphism (wildtype homozygote, heterozygote, variant homozygote) for genotypes *TaqI* and *Apal*, respectively, and their interactions. All final models were adjusted for age, gender, BMI, and vitamin D intake.

Results

The study included 749 participants genotyped for *BsmI* and *TaqI*, and 734 participants for *Apal* polymorphism. Hardy-Weinberg equilibrium (HWE) was assessed prior to data analysis. The vitamin D receptor genotype *BsmI* in the control group (without T2D) was not in HWE and, therefore, was excluded from further analysis. *TaqI* and *Apal* for those without T2D were in HWE. Table 1 presents descriptive statistics by T2D status. Participants with T2D were older ($p < 0.001$), had higher BMI ($p < 0.001$) and lower serum 25(OH)D ($p < 0.001$) levels than did those without T2D. There were no significant differences in combined genotype frequency of *TaqI* and *Apal* by diabetes status.

Table 2 shows the descriptive statistics by VDR genotypes. Distribution of *TaqI* and *ApaI* genotypes differed by ethnicity ($p < 0.001$), with Cuban Americans having a higher percentage of variant homozygosity (12.4%), compared to African Americans (6.5%) and Haitian Americans (5.5%). Only the *TaqI* SNP showed a statistically significant difference between participants with sufficient and insufficient 25(OH)D levels ($p = 0.05$), with only 6.9% of the insufficient being variant homozygote compared to 12.6% of the sufficient.

Table 3 shows descriptive statistics within each ethnicity by vitamin D status. Cuban Americans who were vitamin D insufficient were significantly older ($p < 0.001$) and had a higher percentage of T2D ($p < 0.001$), compared with the sufficient group. *TaqI* genotypes were significantly associated with vitamin D levels ($p = 0.01$), with a smaller percentage (8.5%) of those who were vitamin D insufficient carrying the variant homozygote genotype than those who were sufficient (18.6%). Haitian Americans in the vitamin D insufficient group had a lower proportion of T2D ($p = 0.04$) than did the vitamin D sufficient group. African Americans in the vitamin D insufficient group were significantly younger ($p = 0.04$), had a greater percentage of females ($p = 0.01$), higher BMI ($p < 0.01$), and higher percentage of T2D ($p = 0.03$) than did the vitamin D sufficient group.

The relationship of *TaqI* distribution in Cuban Americans was examined by diabetes status separately, and a significant difference was found in those with T2D ($p = 0.02$). Figure 1 shows the distribution of vitamin D status across VDR genotypes among Cuban Americans with T2D. The *TaqI* variant homozygosity had the lowest frequency in the participants with vitamin D insufficiency, compared to the wildtype homozygosity and

heterozygosity ($p < 0.05$). An unadjusted odds ratio indicated that Cuban Americans with T2D were 5.4 times more likely to be vitamin D insufficient, 95% CI (1.6 to 18.7) if they had TT and Tt. The odds further increased to 7.3 95% CI (2.0 to 26.9) ($p = 0.003$) after controlling for age, gender and BMI.

Discussion

There was no direct association between *TaqI* and *ApaI* polymorphisms with diabetes status. *TaqI* genotype frequencies were marginally significantly different between vitamin D sufficient and insufficient subjects. Additionally, ethnicity was significantly associated with both *TaqI* and *ApaI* genotypes. Our main significant finding was the frequency of the *TaqI* variant homozygote (tt) association with vitamin D levels, where a smaller percentage of those who were vitamin D insufficient carried (tt), as compared to those who were sufficient. Hence, it appears that the (tt) genotype in Cuban Americans may be protective against vitamin D insufficiency. Although this mechanism is still unclear, prior studies have indicated the ability of this SNP to alter transcriptional activity and mRNA stability, thereby altering the abundance of VDR and vitamin D levels (Howard et al., 1995; Morrison, 1994; Orton et al., 2008). However, Vélayoudom-Céphise et al. (2011) found the SNP *FokI* variant allele and the SNP *ApaI* variant homozygosity to be protective against vitamin D deficiency. Additionally, Ogunkolade et al. (2002) found the *TaqI* variant allele to increase insulin secretory capacity in Bangladeshi Asians who reside in the United Kingdom. This finding is important because it correlates with the function of the β -cells of the pancreas, which is associated with vitamin D and T2D. Why and how the differing variant homozygosity offers protection

only in Cuban Americans in our cohort for those with T2D is unclear at present. It may be that environmental factors affect the function of the VDR gene. This finding also may be a chance finding.

Cuban Americans with 25(OH)D insufficiency were also older and had T2D. Age has been found to affect the amount of UVB waves that are emitted into the skin and the rate at which vitamin D is synthesized (Bischoff-Ferrari et al., 2006). Older adults are at particular risk of vitamin D deficiency because, as the skin ages, it cannot synthesize vitamin D efficiently, and the kidneys are less able to convert vitamin D to its active hormone form of 1,25 (OH)₂D (Need, 1993). The role of vitamin D insufficiency in developing T2D has been studied. In Cuban Americans, we found that serum 25(OH)D levels were significantly lower in those with T2D compared to those without. This is in accordance with some, but not all, prior literature that indicates an inverse association between those with diabetes or at risk for diabetes and 25(OH)D levels (Grimnes, 2010; Kayaniyil et al., 2010; Liu et al., 2010; Scragg, Sowers, & Bell, 2004).

African Americans who were vitamin D insufficient were females, had a higher BMI, were younger in age, and a greater percentage had T2D. Increased BMI may result in vitamin D deficiency primarily due to vitamin D being sequestered in fat cells, which leads to lower circulating vitamin D levels (Martini & Wood, 2006). Forrest et al. (2011) found that the rate of vitamin D deficiency doubled in obese adults compared to those in the normal BMI range. Our finding of gender difference is in agreement with that of Maggio et al. (2005), who showed that females had lower plasma 25(OH)D than did males. They hypothesized that females had decreased 25(OH)D earlier in life than did men and that skin thinning, which is a consequence of menopause and is age-related, is

responsible for lowering serum 25(OH)D levels through the action of UVB on 7-dehydrocholesterol. Decreased intake of vitamin D sources such as milk may affect vitamin D levels. This is seen in African American women, who were reported to experience milk as having a negative taste and as being associated with intolerance (Zablah, Reed, Hegsted, & Keenan, 1999). Finally, we found that African Americans with lower 25(OH)D levels were younger. It is possible that younger African Americans spend more time indoors, with less exposure to sunlight, or perhaps are less likely to use supplements, compared to older African Americans.

When we assessed the independent association between vitamin D levels and the VDR gene in both the Haitian Americans and African Americans, no association was noted. It is possible that *TaqI* and *Apal* genotypes do not play an important role in these ethnicities or that a greater sample size for each VDR polymorphism within ethnicities was needed. Factors that may influence the altered vitamin D levels in Haitian Americans and African Americans are the degree of sun exposure, dietary intake, lifestyle factors, and skin color; increased skin pigmentation found in both of these groups can act as a natural sun block and reduce the UVB from penetrating the skin by up to 99% (Holick, 2006). All of these factors may directly or indirectly affect vitamin D levels.

Our findings are specific to three ethnic groups who reside in South Florida and cannot be generalized to these ethnic groups who live in the rest of the U.S. Additionally, the cause-and-effect relationship of vitamin D insufficiency and diabetes status could not be assessed due to the cross-sectional nature of this study. Another limitation is that skin color and parathyroid hormone levels were not assessed.

The main strength of this study was the inclusion of three ethnic groups, providing valuable information on vitamin D levels and VDR polymorphisms in different vitamin D and diabetes status. To our knowledge, the current study is the first to show that there is no association among *Apal*, T2D, and vitamin D levels in Cuban Americans, Haitian Americans, or African Americans, despite having statistical power; however, *TaqI* SNP was associated with vitamin D levels in Cuban Americans.

Further work is needed to assess vitamin D levels in different ethnic groups and the mechanism by which VDR polymorphisms interact with vitamin D and T2D. Future studies also could explore the role in which the vitamin D binding protein and other vitamin D coenzymes interact with vitamin D, VDR, and diabetes status.

In conclusion, we found an inverse association between T2D and vitamin D insufficiency in both Cuban Americans and African Americans. It is still unclear, however, which precedes the other due to the cross-sectional nature of this research. The evidence indicated that the *TaqI* variant homozygote genotype may be protective against vitamin D insufficiency in Cuban Americans with T2D but not in Haitian Americans or African Americans. This is of importance, as it highlights the protective role that VDR polymorphisms may play in relation to vitamin D levels, at least in some ethnicities. Further work is needed to explore how other potential VDR SNPs may influence or enhance vitamin D levels. Our findings should be confirmed through further research in these and other ethnicities.

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Table 1. Characteristics of study participants by diabetes status

Variables	Participants		<i>p</i>
	without T2D (<i>n</i> = 384)	with T2D (<i>n</i> = 365)	
Age (years)	56.1 ± 11.2	59.1 ± 11.5	<0.001
Gender (M) (%; <i>n</i>)	43.5 (167)	43.8 (160)	0.94
BMI (kg/m ²)	30.0 ± 5.6	32.0 ± 6.7	<0.001
Ethnicity (%; <i>n</i>)			0.23
Cuban Americans	38.5 (148)	32.6 (119)	
Haitian Americans	31.0 (119)	34.5 (126)	
African Americans	30.5 (117)	32.9 (120)	
Vitamin D intake (≥600 IU/d) (%; <i>n</i>)	29.9 (115)	25.5 (93)	0.17
Serum 25(OH)D Insufficient (yes) (%; <i>n</i>)	70.6 (271)	80.8 (295)	<0.001
<i>BsmI</i>			<0.001
BB (%; <i>n</i>)	13.5 (52)	6.3 (23)	
Bb (%; <i>n</i>)	65.4 (251)	66.0 (241)	
bb (%; <i>n</i>)	21.2 (81)	27.7 (101)	
<i>TaqI</i>			0.16
TT (%; <i>n</i>)	43.8 (168)	50.7 (185)	
Tt (%; <i>n</i>)	47.4 (182)	41.6 (152)	
tt (%; <i>n</i>)	8.9 (34)	7.7 (28)	
<i>ApaI</i>			0.81
AA (%; <i>n</i>)	36.0 (136)	33.9 (121)	
Aa (%; <i>n</i>)	47.9 (181)	48.7 (174)	
aa (%; <i>n</i>)	16.1 (61)	17.4 (62)	

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05.

Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D.

Table 2. Characteristics of study participants by vitamin D receptor genotypes

Variables	<i>TaqI</i>			<i>p</i>	<i>Apal</i>			<i>p</i>
	TT (<i>n</i> = 253)	Tt (<i>n</i> = 334)	tt (<i>n</i> = 62)		AA (<i>n</i> = 256)	Aa (<i>n</i> = 355)	aa (<i>n</i> = 123)	
Age (years)	57.6 ± 11.8	57.3 ± 11.2	58.8 ± 11.4	0.65	56.1 ± 11	58.3 ± 11.1	58.1 ± 13.3	0.54
Gender (M) (%; <i>n</i>)	48.6 (159)	42.8 (28)	8.6 (140)	0.69	38.4 (123)	47.8 (153)	13.8 (44)	0.08
BMI (kg/m ²)	31.4 ± 6.8	31.2 ± 6.7	29.5 ± 5.1	0.11	31.0 ± 6.8	31.0 ± 6.7	31.9 ± 6.2	0.45
Ethnicity (%; <i>n</i>)				<0.001				<0.001
Cuban Americans	37.8 (101)	49.8 (133)	12.4 (33)		26.8 (69)	50.2 (129)	23.0 (59)	
Haitian Americans	52.7 (129)	40.8 (100)	6.5 (16)		8.4 (93)	51.2 (124)	10.3 (25)	
African Americans	51.9 (123)	42.6 (101)	5.5 (13)		40.3 (95)	43.2 (102)	16.5 (39)	
With T2D (%; <i>n</i>)	50.7 (185)	41.6 (152)	7.7 (28)	0.16	33.9 (121)	48.7 (174)	17.4 (62)	0.81
Vitamin D intake (≥600 IU/d) (%; <i>n</i>)	42.3 (88)	47.1 (98)	10.6 (22)	0.16	35.6 (72)	52.0 (105)	12.4 (25)	0.32
Serum 25(OH)D (nmol/L)	61 ± 28	62.5 ± 26	70.1 ± 33	0.51	60.0 ± 24	63.0 ± 28	64.0 ± 34	0.26
25(OH)D (nmol/L)								
Insufficient				0.05				0.48
Yes (%; <i>n</i>)	48.4 (274)	44.7 (253)	6.9 (39)		36.0 (200)	47.9 (266)	16.0 (89)	
No (%; <i>n</i>)	43.2(79)	44.3 (81)	12.6(23)		31.7 (57)	49.4 (89)	18.9 (34)	

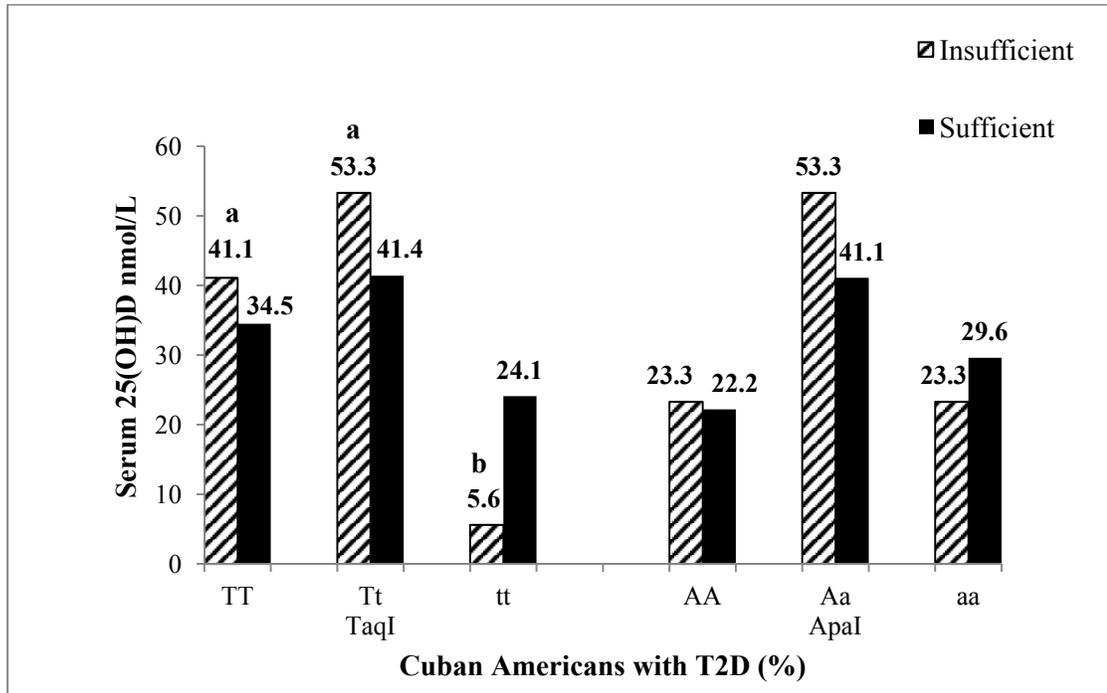
Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D.

Table 3. Characteristics of study participants within three ethnicities by vitamin D status

Variables	Cuban Americans			Haitian Americans			African Americans		
	Insufficient (n = 165)	Sufficient (n = 102)	<i>p</i>	Insufficient (n = 204)	Sufficient (n = 41)	<i>p</i>	Insufficient (n = 197)	Sufficient (n = 40)	<i>p</i>
Age (years)	65.3 ± 11.8	60.1 ± 9.7	<0.001	55.7 ± 9.4	58.3 ± 10.0	0.16	51.9 ± 9.4	55.4 ± 9.7	0.04
Gender (M) (%; n)	30.9 (51)	42.2 (43)	0.06	47.5 (97)	43.9 (18)	0.67	6.2 (91)	67.5 (27)	0.01
BMI (kg/m ²)	31.0 ± 3.0	30.6 ± 5.3	0.39	29.3 ± 5.1	28.3 ± 5.0	0.22	34.3 ± 8.0	29.0 ± 5.9	<0.001
With T2D (%; n)	54.5 (90)	28.4 (29)	<0.001	48.5 (99)	65.9 (27)	0.04	53.8 (106)	35.0 (14)	0.03
Vitamin D intake (≥600 IU/d) (%; n)	27.3 (45)	30.4 (31)	0.58	17.2 (35)	29.3 (12)	0.07	34.0 (67)	45.0 (18)	0.186
<i>TaqI</i>			0.03			0.51			0.94
(TT) (%; n)	37.0 (61)	39.2 (40)		53.9 (110)	46.3 (19)		52.3 (103)	50.0 (20)	
(Tt) (%; n)	54.5 (90)	42.2 (43)		39.2 (80)	48.8 (20)		42.1 (83)	45.0 (18)	
(tt) (%; n)	8.5 (14)	18.6 (19)		6.9 (14)	4.9 (2)		5.6 (11)	5.0 (2)	
Wildtype			0.71			0.38			0.86
homozygote									
TT (%; n)	37.0 (61)	39.2 (40)		53.9 (110)	46.3 (19)		52.3 (103)	50.0 (2)	
Tt/tt (%; n)	63.0 (104)	60.8 (62)		46.1 (94)	53.7 (22)		47.7 (94)	50.0 (20)	
Variant homozygote			0.01			0.64			0.88
tt (%; n)	8.5 (14)	18.6 (19)		6.9 (14)	4.9 (2)		5.6 (11)	5.0 (2)	
TT/Tt (%; n)	91.5 (151)	81.4 (83)		93.1 (190)	95.1 (39)		94.0 (186)	95.0 (38)	

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; 25(OH)D = 25-hydroxyvitamin D.

Figure 1. Distribution of vitamin D status across *TaqI* and *ApaI* VDR gene among Cuban Americans with T2D ($n = 119$)



Serum 25(OH)D Insufficient percentages with different superscripts ^{a,b} were significantly different using Holm's modified Bonferroni method which was used to correct for multiple comparisons. p is considered significant at < 0.05 .

**V. HOMA2 MODEL, VITAMIN D LEVELS, AND DIABETES STATUS
IN THREE ETHNIC GROUPS**

Abstract

Aim: To assess the association among diabetes status, homeostasis model assessment 2 (HOMA2), and vitamin D status in Cuban Americans, Haitian Americans, and African Americans.

Methods: The study included a total of 519 participants, 298 without type 2 diabetes (T2D) and 221 participants with T2D from Florida, U.S. Participants were recruited through mailing lists (African Americans and Cuban Americans) and community-based sources (Haitian Americans). Information was obtained on participants' sociodemographic data, smoking history, and medication(s) used. Height and weight were measured using a SECA balance scale (Seca Corp., Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m². Dietary intake was measured using a semi-quantitative food frequency questionnaire. Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). The homeostasis model assessment (HOMA2) was used to estimate insulin resistance (HOMA2-IR), insulin sensitivity (HOMA2-IS), and β -cell function (HOMA2- β). This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10).

Results: Using the data from the entire cohort, the results indicated that a greater percentage of participants who had T2D were vitamin D insufficient as compared to those without T2D ($p < 0.001$). Within the ethnic groups, distributions of the sufficient and insufficient vitamin D levels were significantly different ($p < 0.001$) by diabetes

status. Among participants without T2D who had sufficient vitamin D levels, Cuban Americans had the highest HOMA2- β , which was significantly higher than that among Haitian Americans and African Americans after adjusting for age, gender, and BMI ($p < 0.05$). Additionally, African Americans' HOMA2- β was lower in the vitamin D sufficient group than in the vitamin D insufficient group ($p < 0.05$).

Conclusion: Cuban Americans without T2D and with sufficient vitamin D levels had significantly higher β -cell function than did vitamin D sufficient Haitian Americans and African Americans. The high percentage of sufficient serum vitamin D levels found in Cuban Americans may explain the better β -cell function in this ethnicity. These data suggest a role for increased vitamin D levels (through supplementation) to potentially improve β -cell function (Mitri, Dawson-Hughes, Hu, & Pittas, 2011). Interestingly, African Americans with vitamin D insufficiency also had increased β -cell function. The inverse associations may have occurred by chance, as the number of participants in the vitamin D sufficient group ($n = 7$) was much smaller than that of the insufficient group ($n = 67$). Further studies, using larger samples, are needed to determine whether these findings remain true.

Introduction

Vitamin D deficiency has been identified as a risk factor for type 2 diabetes (T2D) and cardiovascular disease (Chiu, 2004). While numerous studies have identified an inverse relationship between serum 25(OH)D and both beta cell function and insulin resistance (Forouhi, Luan, Cooper, Boucher, & Wareham, 2008; Nesby-O'Dell et al., 2002; Scragg et al., 2004), few studies have assessed this association in different ethnic minorities who reside in the US. Between 2007 and 2009, 7.1% of Whites, 11.8% of Hispanics (7.6% of Cuban Americans), and 12.6% of Blacks, including African Americans and Haitian Americans, were diagnosed with diabetes (CDC, 2010). Additionally, Scragg et al. (2004) found that 82.1% of Blacks and 69.2% of Hispanics were vitamin D insufficient and that an inverse association between vitamin D levels and diabetes existed only in Whites and Mexican Americans but not in African Americans.

The use of the homeostatic model assessment 2 (HOMA2), based on the Oxford university HOMA2 calculator (www.ocdem.ox.ac.uk) (Wallace, Levy, & Matthews, 2004), is used as a surrogate marker to estimate β -cell function (HOMA2- β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS). Due to the increased prevalence of hypovitaminosis and T2D in certain ethnicities (CDC, 2010; Scragg et al., 2004), there is an increased need to assess the association among vitamin D levels, T2D status, and HOMA2 in ethnic groups that may exhibit differing metabolic characteristics. Therefore, it is the aim of this paper to assess the association among diabetes status, HOMA2 model, and vitamin D status in Cuban Americans, African Americans, and Haitian Americans.

Methods

Participants

In this cross-sectional study, data were from three ethnic minority groups, with ($n = 221$) and without ($n = 298$) T2D, aged 35 years and older.

Data Collection

Participants were initially recruited by random selection from randomly generated mailing lists, purchased from Knowledge Base Marketing, Inc., Richardson, TX, U.S. Ten thousand letters, along with an invitation flyer in both English and Spanish, were mailed to Cuban Americans. Of the participants who received letters, 4% ($n = 388$) responded. Additionally, approximately 7,550 letters were mailed to African Americans with and without T2D. Of the participants who received letters, again 4% ($n = 256$) responded. Because we did not have a similar mailing list database for Haitian Americans, we recruited these participants from community-based sources ($n = 259$): (a) local diabetes educators and community health practitioners in Miami-Dade and Broward Counties; (b) Florida International University faculty, staff, and students; (c) several active adult apartment complexes; and (d) print advertisements and radio advertisements. When the calculated sample size for each ethnic group yielded sufficient power to detect significance differences between major variables, the recruitment of participants was discontinued.

Interested participants were interviewed by phone, at which time the study purpose was explained and age, self-identified ethnicity (Cuban American, Haitian American, and African American), gender, self-reported T2D status (confirmed by

fasting plasma glucose (>126 mg/dL) and/or A1c ($<6.5\%$), years since diagnosis, and initial treatment modalities (oral glucose-lowering drugs and insulin) were confirmed. Participants who did not qualify for the study ($n = 28$) were younger than 35 years old ($n = 12$), of another ethnicity ($n = 5$), or had a chronic disease or illness such as cancer, HIV infection, kidney failure, or hepatitis ($n = 11$), as these conditions could influence the main outcome of the study. This study was approved by the Institutional Review Board at Florida International University (Approval No. 011210-10). Written consent in English, Spanish, or Creole was obtained from the participants on their first visit to the laboratory. A total of 19 participants (Haitian Americans = 8; Cuban Americans = 7; and African Americans = 4) who reported not having diabetes were reclassified in the study as having T2D according to American Diabetes Association (ADA) standards. These participants were provided with a copy of their laboratory results and referred to their physicians. Participants with insufficient amounts of blood serum, out-of-range coefficient of variance, or missing or out-of-range VDR values were excluded. A total of 519 participants (Cuban Americans = 151; Haitian Americans = 185; African Americans = 183) were included in the data analysis.

Measures

Participants were asked to complete standard self-administered questionnaires on site. For detailed questionnaires, trained interviewers were present to interview the participants and help them to complete the questionnaire. Data on participants' sociodemographic characteristics, smoking history, and medication(s) used were obtained. Height and weight were measured using a SECA balance scale with a

stadiometer (Seca Corp., Columbia, MD, U.S.). Body mass index (BMI) was calculated as weight in kg/height in m².

Blood Collection

Twenty ml of venous blood were collected from participants after an overnight fast (at least 8 hours) by a certified phlebotomist using standard laboratory techniques. Blood samples were collected in two tubes: a Vacutainer Serum Separator Tube (SST) for analysis of glucose and another tube containing ethylenediamine tetra-acetic acid (EDTA) for analysis of glycosylated hemoglobin (A1c). After complete coagulation (30-45 minutes), the SST sample was centrifuged at 2,500 RPM for 30 minutes. The serum was transferred into a labeled plastic tube and stored at -70°C until used.

Determination of glucose and vitamin D levels and insulin

Glucose levels were measured by hexokinase enzymatic methods. A1c percentages were measured from whole blood samples with the Roche Tina Quant method by Laboratory Corporation of America (LabCorp, Miami, FL, U.S.). Serum 25(OH)D levels were determined using a commercial ELISA kit from Immunodiagnostic Systems Limited (Scottsdale, AZ, U.S.). The manufacturer's protocol was followed to perform this competitive assay, according to which the standards and samples were diluted with biotin-labeled 25(OH)D. For this study, the interassay coefficient of variation (CV) for ELISA was accepted at less than 10.3% (Bodnar et al., 2007). The cutoff point used to determine insufficient serum 25(OH)D levels was <75 nmol/L (IOM, 2011). Serum insulin levels were determined using the Human Insulin ELISA kit from

Millipore (St Charles, MZ, U.S.). The manufacturer's protocol was followed to perform this Sandwich ELISA assay.

Determination of beta cell function (HOMA2- β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS)

HOMA2 is based on the Oxford University HOMA2 calculator (www.ocdem.ox.ac.uk) (Wallace, Levy, & Matthews, 2004) and is used to estimate beta cell function (HOMA2- β), insulin resistance (HOMA2-IR), and insulin sensitivity (HOMA2-IS). The HOMA2 computer model accounts for variations in hepatic and peripheral glucose resistance (Rudenski, Matthews, Levy, & Turner, 1991). The computer model can be used to determine β -cell function (%B) insulin sensitivity (%S), and the insulin resistance index from paired fasting plasma glucose and radioimmunoassay insulin across a range of 1-2,200 pmol/l for insulin, and 1-25 mmol/l for glucose (Wallace et al., 2004).

Data Analysis

All statistical analyses were performed using SPSS (Version 18.0, Chicago, IL, U.S.). HOMA2-IS and HOMA2-IR were log transformed to achieve normality. Differences in mean values by ethnicity, diabetes status, and vitamin D status were assessed using the Student's *t*-test and analysis of variance (ANOVA) for numerical values and χ^2 test for categorical variables and analysis of covariance (ANCOVA). This was followed by Fisher's least significant difference (LSD) post-hoc test, which was used to compare mean HOMA2 variables across diabetes status, vitamin D levels, and

ethnicity controlling for age, gender, and BMI. The significance level for all analyses was set at $p < 0.05$.

Results

The study included a total of 519 participants, 298 without and 221 with T2D. Table 1 presents the characteristics of study participants with and without T2D. Participants with T2D had a greater percentage of vitamin D insufficiency (83.7%) than those without T2D (67.1%) ($p < 0.001$).

Table 2 presents the characteristics of study participants by diabetes status further categorized into vitamin D insufficient and sufficient. Ethnicity distribution was significantly different between vitamin D sufficient and insufficient participants with and without T2D, with Cuban Americans having a higher percentage of 25(OH)D sufficient participants than did Haitian American or African American participants in both diabetes statuses ($p < 0.01$).

Table 3 presents the characteristics of study participants by diabetes status further categorized by ethnic groups. Participants with T2D and without T2D (wT2D) were analyzed separately. Participants with and without diabetes are not comparable because participants with T2D exhibit different pancreatic functions and altered metabolic functions. The findings showed that Cuban Americans were older than Haitian Americans and African Americans (wT2D, T2D), $p < 0.05$. Cuban Americans, compared to Haitian Americans and African Americans, also had significantly higher means of insulin secretion, HOMA2- β , and log HOMA2-IR (wT2D, T2D), $p < 0.05$. However, Haitian Americans had a significantly higher log HOMA2-IS compared to Cuban

Americans (wT2D, T2D), $p < 0.05$, and African Americans (wT2D, T2D), $p < 0.05$, with the lowest HOMA2- β compared to Cuban Americans, and African Americans (wT2D, T2D), $p < 0.05$. African Americans were significantly younger than Cuban Americans and Haitian Americans (wT2D, T2D) and had the highest BMI compared to Cuban Americans and Haitian Americans (wT2D, T2D), $p < 0.05$.

Table 4 presents the HOMA2- β interaction results between vitamin D status and ethnicity within diabetes status after adjusting for age, gender, and BMI. Interaction between ethnicity and vitamin D level was statistically significant, $F(2, 289) = 3.27$, $p = 0.040$, for participants without T2D. Among participants without T2D who had sufficient vitamin D levels, Cuban Americans had the highest HOMA2- β ($M = 115.1$), which was significantly higher than that for Haitian Americans ($M = 86.2$) and African Americans ($M = 90.4$), $p < 0.05$. For African Americans without T2D, there was a significant difference between vitamin D insufficient and sufficient groups ($p < 0.05$). African Americans with vitamin D insufficiency ($M = 106.9$) had increased HOMA2- β compared to those with vitamin D sufficiency ($M = 90.4$).

Discussion

We have demonstrated that Cuban Americans without T2D and with sufficient vitamin D levels had significantly higher β -cell function than did vitamin D sufficient Haitian Americans and African Americans without T2D. The high percentage of sufficient serum vitamin D levels found in Cuban Americans may explain the higher β -cell function, which suggests the potential role of increased vitamin D levels (through supplementation) in improving β -cell function (Mitri, Dawson-Hughes, Hu, & Pittas,

2011) and where vitamin D is thought to play a direct role on β -cell function (Eliades & Pittas, 2009). The increased levels of vitamin D in Cuban Americans may be a result of greater sun exposure, whereby lighter Caucasian skin pigmentation absorbs 24% of UVB rays compared to Black epidermis, which absorbs 7.4% of UVB (Gloster & Neal, 2006). Altered vitamin D levels also may be associated with genetic factors. Additionally, Cuban Americans without T2D showed increased insulin secretion and higher log HOMA2-IR than did African Americans and Haitian Americans without T2D. The increased log HOMA2-IR in Cuban Americans may be associated with factors such as physical inactivity and an increased BMI (Amati et al, 2009) or being of a specific ethnicity (Haffner et al., 1996).

African Americans without T2D showed a significant difference in β -cell function between participants with and without vitamin D insufficiency, whereby those with vitamin D insufficiency had increased β -cell function. Our findings conflict with those of Chiu, Chu, Liang, Go, and Saad (2004), who found that low levels of vitamin D had a negative effect on β -cell function. They did not, however, have an African American sample as part of their study group. The inverse associations found between vitamin D levels and β -cell function in African Americans may have occurred by chance, as the number of participants in the vitamin D sufficient group ($n = 7$) were much smaller than the insufficient group ($n = 67$). Further studies, using larger samples, are needed to assess whether these findings remain true.

Among the three ethnicities, Cuban Americans and African Americans had higher BMI (>30), β -cell function, log HOMA2-IR, and insulin secretion, with lowest log HOMA2-IS, compared to Haitian Americans. Research has shown that participants

without T2D and with a BMI > 30 are characterized by extra-hepatic insulin resistance, as in seen in Cuban American and African American participants (Felber, 1992; Paquot, 2002). It is important to note that no statistically significant association was seen between β -cell function and BMI in our study. Thus, to draw further conclusions, additional studies should be conducted. Cuban Americans and African Americans also showed an overall decline of HOMA2- β and log HOMA2- IS for those with T2D. This decline demonstrates the nature of T2D, an exhaustion of the β -cells of the pancreas due to chronic insulin resistance (Buchanan, 2002).

The heightened insulin sensitivity in Haitian Americans may be indicative of the role that a lower BMI (<30) may play, whereby a lower BMI increases insulin sensitivity, which leads to lowered insulin secretion and insulin resistance (Ahre'n & Pacini, 2005; Mari, Ahre'n, & Pacini, 2005). The exact mechanism in Haitian Americans who have heightened insulin sensitivity is still unclear. A high percentage of Haitian Americans also had low vitamin D levels; however, these low levels did not play a significant role in log HOMA2-IS, HOMA2- β or log HOMA2- IR. Low circulating levels of 25(OH)D in the majority of Haitian Americans may account for why there was no association was found between vitamin D and the HOMA2 model. It is suspected that low 25(OH)D is primarily due to Haitian Americans' higher skin pigmentation, which causes darker skin color that limits the amount of ultraviolet light that can penetrate, thereby reducing cutaneous synthesis of vitamin D3 (Aloia, 2008). Although Haitian Americans presented unique findings compared to their counterparts, no statistically significant differences were found. This likely is explained by the small percentage of vitamin D sufficient

participants, which decreased the possibility of significant findings. Genetic and other lifestyle factors also underlie the unique findings in Haitian Americans.

The limitations of this study were that skin color was not measured and reverse causation could not be assessed with regard to HOMA2 findings and vitamin D levels. Additionally, β -cell function, among other factors, was calculated using HOMA2, an indirect method of measurement of β -cell function. Another limitation is that few studies exist on the three ethnicities, and, thus, few conclusions and comparisons could be made.

The strengths of the study include its assessment of vitamin D levels with regard to HOMA2 model in three distinct ethnic groups that have been understudied. Additionally, the separation of two Black ethnicities was important, allowing ethnic differences to be identified between African American and Haitian Americans.

In conclusion, participants with and without T2D showed differences due to ethnicity with regard to HOMA2, vitamin D, and general characteristics. These findings indicate that all three ethnicities should be assessed as individual ethnic groups when considering the HOMA2 model. We also can assume that, due to the diverse background of participants in each ethnic group, each presents unique metabolic functions, genetics, diets, and lifestyle factors that may ultimately affect vitamin D levels and progression of T2D. Further investigation is warranted to assess how β -cell function, insulin sensitivity, and insulin resistance may alter in the presence and absence of vitamin D in differing ethnicities.

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Table 1. Characteristics of study participants by diabetes status

Variables	Participants		<i>p</i>
	without T2D (<i>n</i> = 298)	with T2D (<i>n</i> = 221)	
Age (years)	54.4 ± 10.6	60.6 ± 11.7	< 0.001
Gender (F) (%)	53.7 (160)	59.7 (132)	0.170
BMI (kg/m ²)	30.1 ± 5.6	32.5 ± 7.2	< 0.001
Ethnicity (%)			0.068
Cuban American	25.2 (75)	34.4 (76)	
Haitian American	38.3 (114)	32.1 (71)	
African American	36.6 (109)	33.5 (74)	
FPG	97.8 ± 15.0	141.0 ± 53.4	< 0.001
Insulin	11.2 ± 6.9	13.3 ± 9.4	0.006
HOMA2-β	103.2 ± 37.5	70.0 ± 42.3	< 0.001
HOMA2-IS	92.1 ± 50.5	79.3 ± 49.2	0.004
Log HOMA2-IS	4.4 ± 0.6	4.2 ± 0.6	< 0.001
HOMA2-IR	1.5 ± 0.9	1.8 ± 1.4	< 0.001
Log HOMA2-IR	0.2 ± 0.6	0.4 ± 0.6	< 0.001
25(OH)D (nmol/L) Insufficient (Yes) (%)	67.1 (200)	83.7 (185)	< 0.001

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; HOMA2-β = homeostasis model assessment of β-cell function; IR = Insulin resistance; FPG = fasting plasma glucose; 25(OH)D = 25-hydroxyvitamin D. Serum 25-hydroxyvitamin D insufficiency (<75 nmol/L).

Table 2. Characteristics of study participants without and with type 2 diabetes by vitamin D status

Variables	Participants					
	without T2D			with T2D		
	25(OH)D		<i>p</i>	25(OH)D		<i>p</i>
	Insufficient (<i>n</i> = 200)	Sufficient (<i>n</i> = 98)		Insufficient (<i>n</i> = 185)	Sufficient (<i>n</i> = 36)	
Age (years)	52.9 ± 10.5	57.3 ± 10.4	0.67	60.2 ± 12.2	62.8 ± 9.1	0.69
Gender (F) (%; <i>n</i>)	55.0 (110)	51.0 (50)	0.52	60.5 (112)	55.6 (20)	0.58
BMI (kg/m ²)	30.4 ± 5.8	29.4 ± 4.9	0.13	32.6 ± 7.4	30.9 ± 6.1	0.19
Ethnicity (%; <i>n</i>)			<0.001			<0.05
Cuban American	8.0 (16)	60.2 (59)		30.3 (56)	55.6 (20)	
Haitian American	50.5 (101)	13.3 (13)		33.5 (62)	25.0 (9)	
African American	41.5 (83)	26.5 (26)		36.2 (67)	19.4 (7)	
FPG	98.1 ± 15.8	97.4 ± 11.7	0.70	138.7 ± 53.6	154.8 ± 51.0	0.10
Insulin	11.3 ± 7.1	11.3 ± 6.7	0.99	13.0 ± 9.4	14.5 ± 9.1	0.40
HOMA2-β	103.4 ± 39.2	102.8 ± 34.0	0.92	72.5 ± 43.6	61.7 ± 33.8	0.16
HOMA2-IS	92.8 ± 51.4	90.7 ± 49.0	0.74	82.2 ± 50.8	64.5 ± 37.1	0.01
Log HOMA2-IS	4.4 ± 0.57	4.4 ± 0.6	0.86	54.2 ± 0.7	4.0 ± 0.6	0.11
HOMA2-IR	1.5 ± 0.94	1.5 ± 0.9	0.99	1.9 ± 1.4	2.1 ± 1.3	0.56
Log HOMA2-IR	0.2 ± 0.57	0.2 ± 0.6	0.85	0.4 ± 0.7	0.6 ± 0.6	0.10

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; HOMA2-β = homeostasis model assessment of β-cell function; IR = Insulin resistance; FPG = fasting plasma glucose; 25(OH)D = 25-hydroxyvitamin D. Serum 25-hydroxyvitamin D insufficiency <75 nmol/L.

Table 3. Characteristics of study participants without and with type 2 diabetes by ethnicity

Variables	Participants							
	without T2D (<i>n</i> = 298)				with T2D (<i>n</i> = 221)			
	Cuban Americans (<i>n</i> = 75)	Haitian Americans (<i>n</i> = 114)	African Americans (<i>n</i> = 109)	<i>p</i>	Cuban Americans (<i>n</i> = 76)	Haitian Americans (<i>n</i> = 71)	African Americans (<i>n</i> = 74)	<i>p</i>
Age (years)	60.0 ± 10.7 ^a	54.1 ± 10.9 ^b	50.9 ± 8.5 ^c	<0.001	67.1 ± 11.4 ^a	58.6 ± 10.0 ^b	55.9 ± 10.7 ^c	<0.001
Gender (F) (%; <i>n</i>)	61.3 (46)	51.8 (59)	50.5 (55)	0.302	65.8 (50)	57.7 (41)	55.4 (41)	0.396
BMI (kg/m ²)	30.1 ± 4.2 ^{ab}	28.8 ± 5.0 ^b	31.3 ± 6.6 ^a	0.003	32.6 ± 7.2 ^b	29.2 ± 4.5 ^c	35.2 ± 8.1 ^a	<0.001
FPG	97.1 ± 13.2	99.6 ± 16.7	96.4 ± 12.9	0.233	146 ± 54.5	145.0 ± 51.6	133 ± 53.5	0.261
Insulin	12.5 ± 6.8 ^a	10.0 ± 5.5 ^c	11.7 ± 8.2 ^b	0.033	17.4 ± 10.8 ^a	9.9 ± 7.8 ^c	12.2 ± 7.4 ^b	<0.001
HOMA2-β	111.7 ± 33.1 ^a	94.9 ± 38.7 ^b	106 ± 37.5 ^{ab}	0.006	80.0 ± 44.7 ^a	56.4 ± 37.1 ^c	74.9 ± 41.2 ^b	0.002
HOMA2-IS	80.2 ± 44.2 ^b	99.6 ± 51.4 ^a	92.4 ± 52.5 ^b	0.034	60.1 ± 46.1 ^b	98.9 ± 51.4 ^a	80.2 ± 42.7 ^c	<0.001
Log HOMA2-IS	4.2 ± 0.5 ^b	4.5 ± 0.5 ^a	4.4 ± 0.6 ^{ab}	0.027	3.9 ± 0.6 ^b	4.4 ± 0.6 ^a	4.2 ± 0.6 ^c	<0.001
HOMA2-IR	1.7 ± 0.9 ^a	1.3 ± 0.7 ^b	1.6 ± 1.1 ^{ab}	0.041	2.5 ± 1.5 ^a	1.4 ± 1.1 ^b	1.7 ± 1.1 ^b	<0.001
Log HOMA2-IR	0.4 ± 0.5 ^a	0.1 ± 0.5 ^b	0.2 ± 0.6 ^{ab}	0.029	0.7 ± 0.6 ^a	0.2 ± 0.6 ^c	0.4 ± 0.6 ^b	<0.001
25(OH)D Insufficient (Yes) (%; <i>n</i>)	21.3 (16)	88.6 (101)	76.1 (83)	<0.001	73.7 (56)	87.3 (62)	90.5 (67)	0.012

Data are means ± SD unless otherwise indicated. *p* is considered significant at < 0.05. Abbreviations: T2D = Type 2 diabetes; BMI = body mass index; HOMA2-β = homeostasis model assessment of β-cell function; HOMA2-IS = homeostasis model assessment of insulin sensitivity; HOMA2-IR = homeostasis model assessment of insulin resistance; FPG = fasting plasma glucose; 25(OH)D = 25-hydroxyvitamin D. Serum 25- hydroxyvitamin D insufficiency (<75 nmol/L). Ethnic groups with different superscripts are significantly different from each other. Fisher’s least significant difference (LSD) test was used to correct for multiple comparisons.

Table 4. HOMA2- β means by vitamin D status and ethnicity within diabetes status

Variable	25(OH)D	Cuban American	Haitian American	African American
Without T2D	Insufficient (<75 nmol/L)	100.9 ± 8.8	99.1 ± 3.5	106.9 ± 4.0
	Sufficient (≥75 nmol/L)	115.1 ± 4.6 ^a	86.2 ± 9.6 ^b	90.4 ± 6.8 ^b
With T2D	Insufficient (<75 nmol/L)	82.7 ± 5.9	57.1 ± 5.4	78.0 ± 5.3
	Sufficient (≥75 nmol/L)	65.3 ± 9.1	61.7 ± 13.7	53.1 ± 15.5

Interaction between ethnicity and vitamin D was statistically significant $F(2, 289) = 3.27, p = 0.040$. Data are means ± SD unless otherwise indicated. Control variables included were: age, gender, BMI. p is considered significant at $p < 0.05$. Ethnic groups with different superscripts in a row are significantly different from each other. Fisher’s least significant difference (LSD) test was used to correct for multiple comparisons. Abbreviations: T2D = Type 2 diabetes

VI. SUMMARY AND DISCUSSION

This dissertation examined the association of vitamin D levels, VDR polymorphisms and HOMA2 model in Cuban Americans, Haitian Americans, and African Americans with and without T2D.

We found that for all ethnicities combined that vitamin D insufficiency was greater in participants with T2D than without after controlling for all relevant variables. The mechanism of action of vitamin D in diabetes is not completely understood in humans; however, it is thought that vitamin D deficiency impairs insulin synthesis and secretion in β - cells of the pancreas, resulting in the development of T2D (Gerich, 2003). There were significant differences in vitamin D levels across ethnic groups and diabetes status with Cuban Americans having the highest vitamin D levels compared to Haitian Americans, and African Americans. Scragg et al.(2004) used the Third National Health and Nutrition Examination Survey and found ethnic variations in 25(OH)D levels. Whites had the highest 25(OH)D concentrations followed by Mexican Americans and lowest in Blacks (Scragg et al., 2004). These results are in agreement with our study. The most likely explanation of the vitamin D difference found, may be partially explained by the variation in the skin color by ethnicity. Where, Cuban Americans had predominantly lighter skin color which may have allowed them greater UVB exposure than Haitian Americans, and African Americans. Our hypothesis that Cuban American, Haitian American, and African American participants combined, with T2D will have lower serum vitamin D levels than their counterparts without T2D is based on research of Baynes et al.(1997) and was found to be highly significant. However, upon stratification of ethnicities, this finding was only significant in Cuban Americans. Haitian Americans

without T2D showed greatest risk of 25(OH)D insufficiency when compared to Cuban Americans without T2D. Factors such as skin pigmentation, genetic or lifestyle factors may have contributed to the Haitian American findings.

Our study supports the hypothesis that Haitian American, and African American participants will have lower serum vitamin D levels as compared to Cuban American, in particular, Cuban Americans without T2D. Our findings did not support the hypothesis that the Cuban Americans, Haitian Americans, and African Americans with adequate (>400 IU/day) vitamin D intake will have ‘sufficient’ ($\geq 75\text{nmol/L}$) serum vitamin D levels regardless of diabetes status. We found that African Americans had the highest vitamin D intake (~ 449 IU/day) based upon 400IU/day, which was the old criterion for recommended dietary allowance of vitamin D. However, this intake did not translate into sufficient serum vitamin D levels in this population group. In 2010 the Institute of Medicine (IOM) defined recommended dietary allowance of vitamin D as 600 IU/day for those between the ages of 1-70 (including pregnancy and lactation), and 800 IU/day in those over 70 (IOM, 2010). Regardless of the altered intake criteria, analysis of this population group still would have an average intake of ~ 449 IU/day in African Americans, which would be below the newly recommended 600 IU/day from the IOM but still not at a ‘sufficient’ level in order to support our hypothesis.

When we assessed the relationship among serum 25(OH)D levels, VDR polymorphisms (*BsmI*, *TaqI* and *Apal*), and diabetes status in the three ethnic groups (Cuban Americans, Haitian Americans, and African Americans) residing in South Florida, we found that *BsmI* (rs1544410) genotype distribution showed a departure from the Hardy-Weinberg equilibrium (HWE) due to the low frequency of the wildtype

homozygote genotype and an excess of heterozygosity in groups with and without T2D. *BsmI* was excluded from the study on the basis that results on the agarose gel and in TaqMan allelic discrimination assay by real-time PCR were inconsistent and unclear. Before we ruled out *BsmI*, a number of tests were run in order to identify the nature of the deviation from HWE. Firstly, to rule out misclassification of samples (1) gel bands were re-examined by a second researcher with no knowledge of prior interpretation of the bands and diabetes status of samples. To rule out partial digestion of samples, running real time PCR on 5% of randomly selected control samples of each genotype (BB, Bb, and bb) was conducted and compared to TaqMan results. Genotyping results remained ambiguous and indicated a greater heterozygosity rate in PCR-RFLP. It was therefore concluded that either an unknown polymorphism around the SNP or a paralogous gene sequence in the genome interfered with the genotyping of rs1544410. In addition, upon further analysis, we found that *BsmI* and *TaqI* polymorphisms are in strong linkage disequilibrium. Polymorphisms that are in linkage disequilibrium have the same predictive value for the association with the phenotype (Ersoz, Yu, & Buckler, 2009). This means that association results for locus *BsmI* (rs1544410) and *TaqI* (rs7321236) would have been similar. Therefore, only one polymorphism needed to be studied, and thus *BsmI* was safely removed and *TaqI* assumed its place (a detailed account is presented in Appendix I).

We found an association between *TaqI* and *Apal* genotypes among ethnicities ($p < 0.001$). Cuban Americans were 7.3 times more likely to be vitamin D insufficient if they had the recessive trait *TaqI* TT and Tt, 95% CI (1.97 to 26.9). This partially supports the hypothesis that there will be a higher prevalence of polymorphisms in the VDR gene

restriction sites *BsmI*, *ApaI* and *TaqI* for participants with diabetes as compared to participants without diabetes for all ethnicities. As an association between *TaqI* and vitamin D was only found in Cuban Americans with T2D. No association was found in *ApaI*, ethnicity and vitamin D levels. We also found no association between differences in the prevalence and types of VDR polymorphisms among the three ethnicities in both participants with and without T2D. This may be due to the small sample size within each ethnicity and where each VDR polymorphism was further divided into three genotypes.

A number of hypotheses were not proven as no association was found between HOMA2 modules and VDR polymorphism in each ethnicity, this again is believed to be due to the small sample size for VDR polymorphisms. Further work is needed to assess vitamin D levels in different ethnic groups and the mechanism in which VDR polymorphisms interact with vitamin D and T2D.

Finally, we assessed the association among diabetes status, HOMA2 model and vitamin D status in Cuban Americans, Haitian Americans, and African Americans. Cuban Americans had the highest HOMA2- β which was statistically different from both Haitian Americans and African Americans. Additionally, African Americans HOMA2- β was lower in the vitamin D sufficient group compared to the vitamin D insufficient group. We found no significant association between HOMA2-IR and vitamin D levels in for any of the ethnicities, which disproves the hypothesis that an association would be found.

We can conclude that of the three ethnicities, Cuban Americans had the highest vitamin D levels, a protective factor in SNP *TaqI* (tt) for Cuban Americans with T2D, and the highest β -cell function compared to the African Americans and Haitian Americans.

VII. STRENGTHS AND LIMITATIONS

Our study had some significant strengths; firstly it included three different ethnic groups, providing valuable information regarding vitamin D levels, VDR polymorphisms and HOMA2 model in different ethnicities and diabetes status with few other studies focusing on these ethnicities. This study is the first to consider differences in two Black American ethnicities with regards to VDR polymorphisms and HOMA2 model.

There were several limitations of this study; we could not determine cause and affect due to the observational and cross sectional nature of the study. The samples of Cuban Americans, Haitian Americans, and African Americans may not be representative of the same ethnicities residing in other parts of Florida or the U.S. Skin color, parathyroid hormone levels and vitamin D binding protein were not assessed in order to better understand vitamin D status. Additionally, the proportion of subjects that were vitamin D sufficient was small in the Haitian Americans, and African Americans, which may have influenced our results. HOMA2 model was used as a surrogate/indirect marker for insulin sensitivity, resistance and β -cell function; other direct measures could have been used such as C-peptide measurement for β -cell function and euglycaemic insulin clamp for insulin resistance which has been considered the gold standard (Ferrannini & Mari, 1998).

Findings suggest further research is needed to assess vitamin D levels in different ethnic groups, with emphasis on β -cell function, insulin secretion and insulin resistance. Additionally, the mechanism in which VDR polymorphisms interact with vitamin D and T2D is necessary to further understand the complexity that ethnicity combined with chronic disease may present.

VIII. FUTURE RESEARCH

Our findings indicate a strong need to recognize each ethnicity as individual populations with special attention to the differences within Black populations. A greater understanding is needed with regards to the role of vitamin D in the metabolism of glucose, insulin production, and regulation of β -cell function. Cuban Americans, Haitian Americans and African Americans should also be further investigated to have a better understanding of the role of Vitamin D, VDR polymorphisms and HOMA2 model. Awareness of the high prevalence of vitamin D insufficiency among African Americans and Haitian Americans and also in those with T2D may prompt awareness among physicians and dietitians with intent to prevent vitamin D insufficiency.

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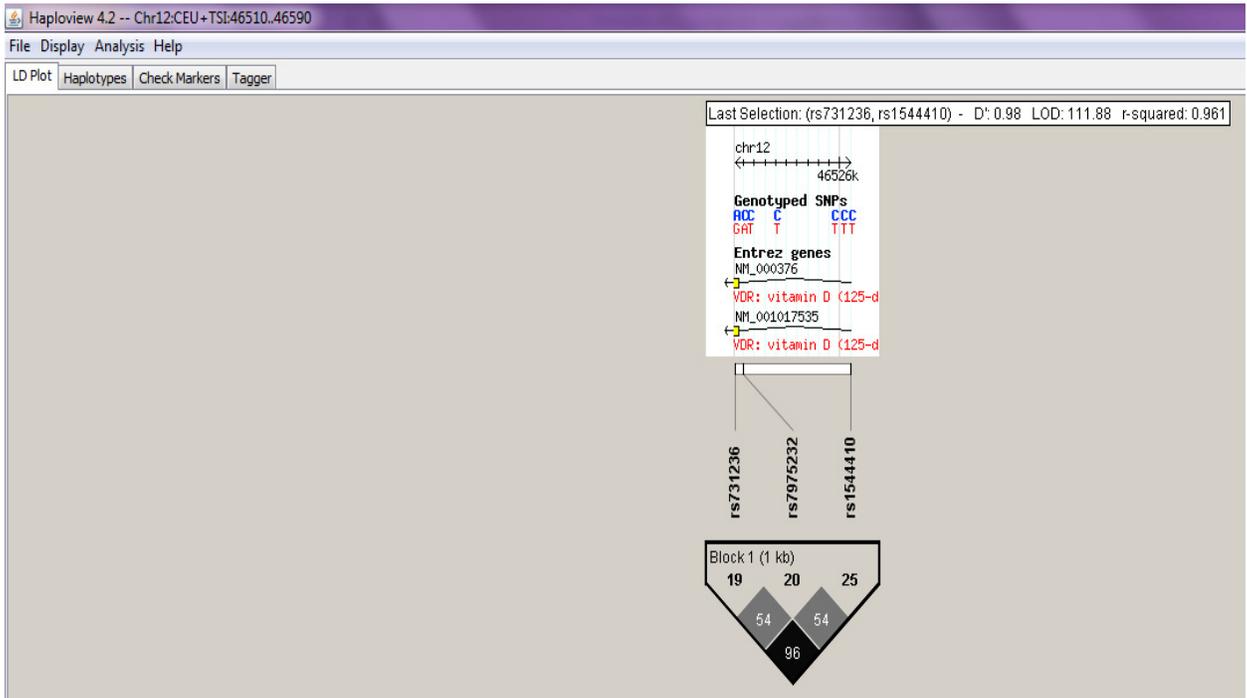
APPENDIX

Table 1. Vitamin D receptor genotype (*BsmI*) by diabetes status.

Variables	Participants		Participants	
	without T2D (%)	<i>p</i> value	with T2D (%)	<i>p</i> value
<i>BsmI</i>				
Cuban American		<0.05		<0.05
<i>BB</i>	20.3		5.9	
<i>Bb</i>	65.5		89.1	
<i>bb</i>	14.2		5.0	
African American		<0.05		0.1
<i>BB</i>	6.0		8.3	
<i>Bb</i>	77.8		51.7	
<i>bb</i>	16.2		40.0	
Haitian American		0.2		0.05
<i>BB</i>	12.6		4.8	
<i>Bb</i>	52.9		57.9	
<i>bb</i>	34.5		37.3	

Abbreviations: T2D = Type 2 diabetes. *p* is considered significant at < 0.05.

Figure 1. Linkage disequilibrium of *BsmI*, *TaqI* and *ApaI*



All three SNPs are highly correlated with one another in Caucasians. Especially, rs731236 (*TaqI*) and rs1544410 (*BsmI*) are in strongest correlation (D' 1.0 out of 1.0; LOD score = 112 (significance threshold is 3.0); $R^2 = 0.96$ out of 1.0 and significance threshold for high correlation is 0.80). This indicates that there is no need to repeat both SNPs (rs731236 and rs1544410) since one of them is providing the same information as the other.

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