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**ASSOCIATION OF VITAMIN D DEFICIENCY WITH
PREDIABETES AMONG ELDERLY NEW MEXICANS**

by

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THESIS

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DEDICATION

Thanks:

To my loving and supportive husband, Raj and my two children, Sumita and Kiran.

To my parents who always has been there for me since I was a child.

To my lifetime role model and inspiration, my uncle Dr. Krishnan.

Association of Vitamin D Deficiency with Prediabetes among Elderly New Mexicans

By

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ABSTRACT

In New Mexico, the mortality rate due to diabetes is 1.5 times higher than the nation. Diabetes causes a huge healthcare burden. In 2012, the national cost due to diabetes was $245 billion.   Prior studies have established a positive correlation between vitamin D deficiency and diabetes; whether the same relationship holds for prediabetes is not well studied. Our purpose of the study was to find whether there was an association between vitamin D deficiency and prediabetes. We also wanted to find out if the association was stronger among Hispanics than non-Hispanic whites (NHW). Our rationale was to delay the progression of diabetes by preventing at a prediabetes stage with vitamin D supplementation in deficient population.   In New Mexico, there are 106,310 adults diagnosed with prediabetes which is 7% of our population.   Therefore, it is crucial that a preventive measure is in place to impede the progression of diabetes among a vulnerable population where there is a high rate of diabetes, obesity and vitamin D deficiency.

We planned to conduct our research with a cross-sectional study design. To do this we randomly selected 200 subjects from New Mexico Elder Health Survey 1993-1995, containing only Medicare recipients of Albuquerque. The participants were 65 years and older, containing non-Hispanic whites (55%) and Hispanics (45%) with relatively equal subjects with normal fasting plasma glucose and prediabetes. There were equal number of males and females. Also there were equal distributions of normal and deficient vitamin D level among our study subjects.

From our statistical analyses, we found there was no association between vitamin D deficiency and prediabetes. However, when we conducted multivariable logistic regression analysis with interaction between Hispanic ethnicity and vitamin D deficiency with prediabetes as outcome, we found that Hispanics with vitamin D deficiency were 2.4 fold increased odds for having prediabetes as compared to NHW.

Our findings are innovative, interesting and novel which will provide rationale for future research on vitamin D and prediabetes.

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CHAPTER 1: INTRODUCTION

Background

Prediabetes

Prediabetes is an early stage in the continuum of diabetes where plasma glucose is elevated but not high enough to be classified as diabetes. Prediabetes is also known as impaired glucose tolerance or impaired fasting glucose. The criteria for diagnosis of prediabetes are based on either fasting plasma glucose (FPG) between 100 mg/dL and 125 mg/dL or hemoglobin A1c level, a measure of average glucose over 2 month period is between 5.7% and 6.4% (1).   In 2010, an estimated 79 million American adults aged 20 years and older were diagnosed with prediabetes. Individuals with prediabetes are up to 5-15 times more likely to develop diabetes than individuals with normal blood glucose levels (2).   According to data from the telephone-based New Mexico Behavioral Risk Factor Surveillance System (BRFSS) survey, 7.0% or 106,310 of New Mexico adults aged 18 and older had diagnosed prediabetes in 2008-2010 (3).

Diabetes

Diabetes is a major public health problem.   Several risk factors including higher body mass index (BMI), family history, diet and sedentary lifestyle habits play a role in the pathogenesis of diabetes. The national cost of diabetes in the U.S. in 2012 exceeds $245 billion constituting a huge health care burden. This includes a direct cost of $176 billion accounting for all medical expenses and an indirect cost of $69 billion for loss of productivity from unemployment and disability due to

diabetes (4). In New Mexico the 8.3% overall rate of diabetes is similar to that of

nation but the mortality rate from diabetes in New Mexico is 1.5 times higher (4).

Recent research indicates vitamin D is intimately related to diabetes. Vitamin D

may delay the progression of diabetes at an early stage (5). National Institute of

Diabetes and Digestive and Kidney Diseases (NIDDK), a division of National

Institute of Health (NIH) has proposed $5 million for the Fiscal Year 2013

Presidential Budget request to a support multicenter study to test whether vitamin

D can prevent diabetes in high risk adults (5).

There are a number of study findings that demonstrate a positive correlation

between vitamin D deficiency and diabetes in humans.   A study by Johnson et

al. suggested the mechanism behind this relationship occurs due to the presence

of vitamin D receptors (VDR) on pancreatic β cells in the islets of Langerhans (6).

In addition, other research supports a direct effect of vitamin D on insulin

synthesis and secretion because of the presence of the vitamin D response

element in the human insulin gene promoter and the transcriptional activity of the

human insulin gene caused by 1, 25-dihydroxyvitamin D, the active form of

vitamin D (7, 8).   A positive correlation has been demonstrated between serum

vitamin D concentration and insulin sensitivity index wherein low serum vitamin D

was associated with increased risk for insulin resistance (9).   Previously studies

have established that a positive correlation exists between vitamin D deficiency

and diabetes. The potential mechanism behind the pathogenesis of diabetes

was described by Pittas et al. after a systematic review analysis of 44 literature

studies involving observational studies and randomized control trials concluded

that there was a direct effect of vitamin D on insulin secretion. The reason was attributed to the mechanism where vitamin D binding to VDRs on β cells causes an influx of calcium through the calcium channels which influences the insulin filled vesicles to be released via exocytosis into plasma to maintain the homeostasis of glucose. They concluded that alteration in vitamin D level impaired the insulin release that caused a disturbance in the homeostasis of plasma glucose resulting in diabetes. Thus it was reasonable to propose that people with type 2 diabetes have low vitamin D as has been suggested by observational study findings (10) (Figure 1).

Fig 1. Potential Mechanism: Pathogenesis of Diabetes

Pittas et al. 2007, Journal of Clinical Endocrin Metab
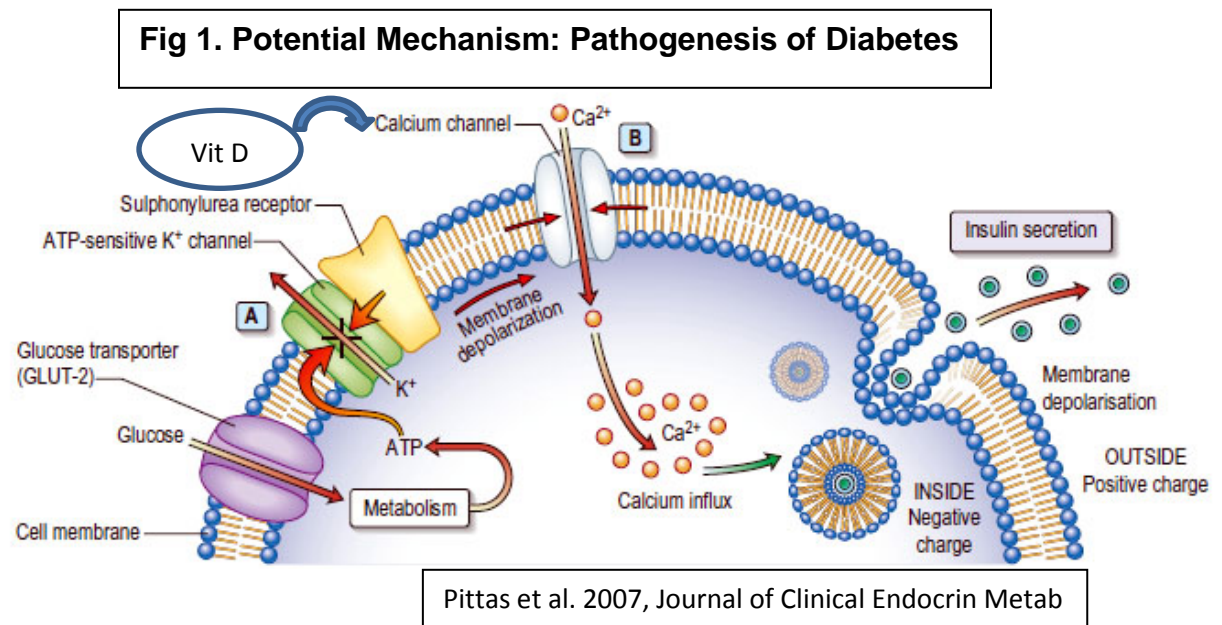
In addition to a direct effect, an indirect effect of vitamin D on the pancreatic β cell may be mediated through its regulation of calcium that in turn affects insulin secretion, which is a calcium dependent process (11). Is there an association of vitamin D deficiency with prediabetes among Elderly New Mexicans? The association between vitamin D deficiency and diabetes is well documented (7, 8,

9, 12).   However, little is known about whether such an association exists with prediabetes. Further, the link between vitamin D deficiency in New Mexico population and diabetes is poorly studied. We proposed a cross-sectional study to evaluate the association between vitamin D deficiency and prediabetes among Hispanic and non-Hispanic white New Mexicans. Our research objective was to determine if there is an association between vitamin D deficiency and prediabetes in our New Mexico population. This population is ideal to carry out this research as New Mexico has a relatively large number of individuals with diabetes (4) and has an ethnically diverse population with equal distribution of Hispanics and non-Hispanic whites (13) and increased rate of vitamin D deficiency (14). Persons of Hispanic ethnicity have been found to have high rates of vitamin D deficiency (15).   If we found an association between vitamin D deficiency and prediabetes then simple preventive measure such as vitamin D supplements might prevent progression to diabetes (16).

Vitamin D Background

It was estimated that 24% of the U.S. population is at risk of vitamin D inadequacy (12-20 ng/mL), and 8% are at risk of vitamin D deficiency ($\leq$ 12 ng/mL) (48) (Fig 2). Vitamin D is a fat soluble vitamin that is primarily synthesized in skin from direct sunlight.

Fig 2 Serum 25OHD status of persons aged 1 year and over: United States, 2001–2006

Possibly harmful (>125 nmol/L) 1%

At risk of deficiency (<30 nmol/L) 8%

At risk of inadequacy (30–49 nmol/L) 24%

Sufficient (50–125 nmol/L) 67%
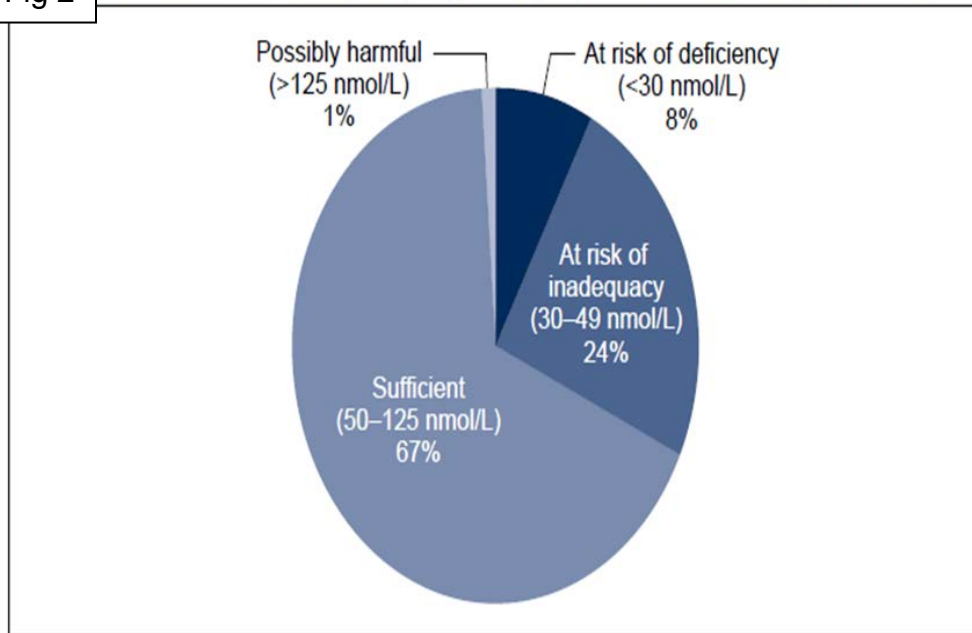
NOTES: 25OHD is 25-hydroxyvitamin D. To convert nmol/L to ng/mL, divide by 2.5.
SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey (NHANES); data for ages 1–5 years from NHANES 2003–2006.

The majority of vitamin D in adults is synthesized in the epidermis of the skin accounting for more than 80% of total previtamin D3 (17). The synthesis of vitamin D occurs when ultraviolet (UV) B radiation with a wavelength of 290-320 nanometers penetrates uncovered skin and converts cutaneous 7-dehydrocholesterol to previtamin D3, which in turn becomes vitamin D3 (11). Only a small amount (30%) is obtained from the diet (12). The best dietary sources of vitamin D are fatty fish such as salmon, tuna and mackerel and fish liver oils. Also small amounts of vitamin D are found in beef liver, cheese, egg yolks and vitamin D fortified foods (18).

It has been suggested by vitamin D researchers that approximately 5-30 minutes of sun exposure between 10 AM and 3 PM at least twice a week to the face,

arms, legs or back without sunscreen usually lead to sufficient vitamin D

synthesis (18).   Vitamin D must go through a series of two hydroxylation

processes to produce the biologically active form of vitamin D, $1,25(OH)_2D$ (19).

The first hydroxylation process occurs in the liver and forms $25(OH)D$ and the

second hydroxylation step occurs in the kidney to form the active vitamin D,

$1,25(OH)_2D$ (Fig 3).   The active form of vitamin D then binds to vitamin D

binding protein and reaches target organs containing vitamin D receptors (20).

In summary, vitamin D receptors are present on β cells and that vitamin D is

essential for pancreatic β cell to secretion of insulin and is responsible for insulin

sensitivity. Therefore, vitamin D deficiency leads to β cell dysfunction that

impairs the homeostasis of plasma glucose.

Fig 3   Vitamin D Synthesis
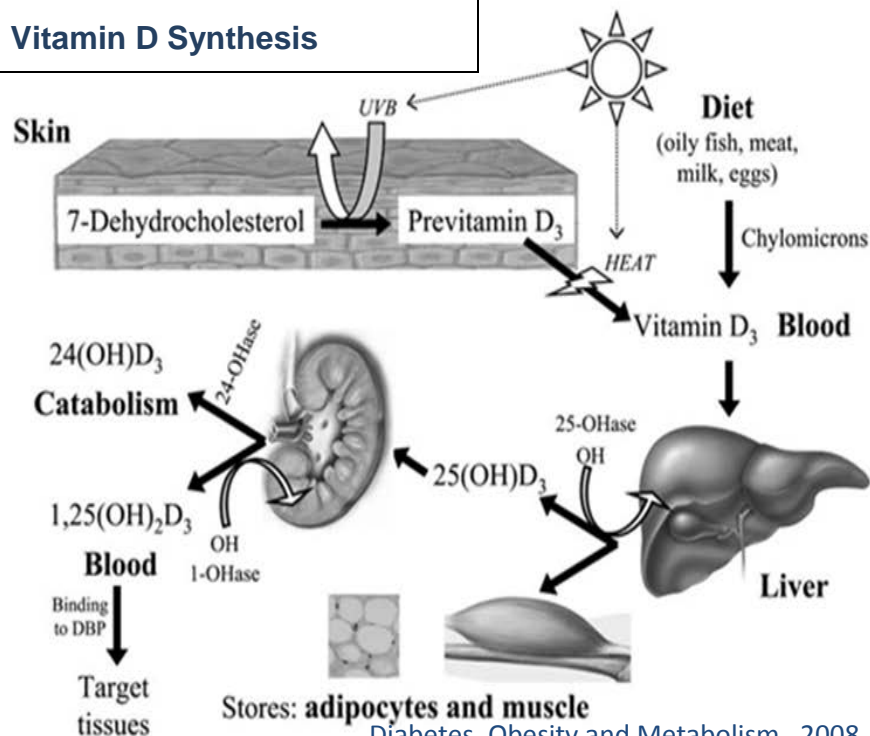
Diabetes, Obesity and Metabolism, 2008

Fig 3 Vitamin D$_3$ may be obtained directly from the diet or by means of the sunlight-induced photochemical conversion of 7-dehydrocholesterol to previtamin D$_3$. Previtamin D$_3$ is thermodynamically unstable and undergoes thermally induced conversion to Vitamin D$_3$. Whatever the source, vitamin D$_3$ must be hydroxylated twice to produce the biologically active form. Thus, the first hydroxylation process takes place in the liver and forms 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) and is cata-lysed by vitamin D-25-hydroxylase (25-OHase). The second hydroxylation step, which produces the final active metabolite of vitamin D$_3$ (1,25(OH)$_2$D$_3$), is mediated by 25-hydroxyvitamin D$_3$ 1α-hydroxylase (1-OHase) and occurs predominantly in the kidney. Then, 1,25(OH)$_2$D$_3$ is released into the circulation where it binds to vitamin D–binding protein (DBP) until it reaches its target tissue by means of the vitamin D receptors (VDR). Vitamin D 24-hydroxylase (24-OHase) is the enzyme which catalyses the catabolism of the hormone in the kidney.

Diagnosis Criteria

The best indicator for vitamin D status is 25(OH)D because it reflects both cutaneous production as well as from food and supplement intake (18) and it has a slower rate of clearance than the active form 1,25(OH)$_2$D (12).

Potential Predictors of Vitamin D Deficiency

1. **Skin type -** The type of skin a person possesses determines the vitamin D level. The amount of previtamin D3 that can be synthesized in the skin from UVB

radiation depends on skin type. According to Fitzpatrick scale (21), fair-skinned individuals (skin types I/II), pale skin maximizes the use of any UV radiation in weak sunlight for vitamin D3 synthesis. Whereas, in dark-skinned individuals (skin types V/VI) (21), only a fraction of the available UVB reaches the skin cells where 7-dehydroxycholesterol (7-DHC) conversion to previtamin D3 occurs (22). Skin types III and IV are intermediate in their response to UV production of vitamin D. An experimental study conducted by Chen et al. demonstrated that the conversion of epidermal 7-DHC to previtamin D3 in Type II skin was approximately 5-10 fold more efficient than highly pigmented type V skin (23). This is due to the fact that melanin in dark skin types absorbs UV radiation preventing the conversion of 7-DHC to previtamin D3 (22).

2. **Ethnicity –** Previous studies have noted that the prevalence of vitamin D deficiency in Hispanics is higher than non-Hispanic whites. For instance, Forrest et al. study demonstrated this by analyzing the NHANES (National Health and Nutritional Examination Survey) data, 2005-2006 to evaluate the vitamin D deficiency in the U.S. population particularly among minority population. They concluded that the prevalence rate of vitamin D deficiency is highest among Blacks (82.1%) followed by Hispanics (62.9%), and lowest in Whites (30%) (14). Other studies have shown that vitamin D levels can differ by ethnicity with Blacks and Hispanics consistently manifesting lower serum concentrations of vitamin D than non-Hispanic whites (15). One possible mechanism is that the darker skin pigmentation of Blacks and Hispanics inhibits cutaneous synthesis of cholecalciferol, the metabolic precursor to vitamin D (25). Analysis of the

National Health and Nutrition Examination Survey (NHANES), 2005 to 2006 data were conducted to measure the overall prevalence rate of vitamin D deficiency. The result from the data analysis found that African American adults had the highest prevalence rate of vitamin D deficiency (82.1%; 95% CI, 76.5%-86.5%), followed by Hispanic adults (62.9%; 95% CI, 53.2%-71.7%) and whites with the lowest prevalence (30% at 95% CI) (14).

3. **Age –** Skin changes with aging. There is an age-dependent decrease in the 7-DHC in the stratum basale (Fig 4), the actively reproducing layer of the epidermis (26). The empirical finding from UV exposure to skin samples of 8 and 18 year olds compared to subjects 77- and 82 year olds revealed a dramatic decline in the ability of the epidermis to produce previtamin D_3. This is attributed to the decreased

Fig 4. Layers of Human Skin

http://spaces.imperial.edu/thomas.morrell/cha_5_tortora_integument.htm

photosynthesizing capacity of previtamin D_3 in the elderly as compared to that of a young person (26). The highest rates of vitamin D deficiency (approximately 48%) were found in the age group between 55 to 64 years as compared to the age group between 30-34 years that had the lowest rates of vitamin D deficiency (approximately 36%) (14).

4. **Obesity** – Obesity also has a role in vitamin D concentration. Obese individuals (BMI≥30) have significantly lower circulating vitamin D than non-obese individuals. To demonstrate this, Blum et al. performed liquid chromatography/mass spectrometry to measure the vitamin D level in the serum and fat tissue of morbidly obese subjects undergoing gastric bypass surgery (27). They found an inversely relationship associated with obesity and vitamin D concentration (27). The inverse association was suggested to be due to the adipose tissue acting as a repository for vitamin D by increased metabolic clearance through enhanced uptake in fat tissue and/or decreased bioavailability of vitamin D once it is deposited in fat tissue (27). Muscogiuri et al. investigated the relationship between 25(OH)D concentration and insulin sensitivity and found a significant correlation between 25(OH)D and BMI ($r=-.58$; $P=001$). In this study BMI was found to be the most powerful predictor of vitamin D with a correlation of -0.52, $P<0.01$) (28).

Gaps in knowledge and rationale of this study

This transition from prediabetes to diabetes may be due to either insulin resistance and/or impaired beta cell function secreting insulin. Vitamin D deficiency is related to the factors such as skin types, older age, and obesity have been linked to incident diabetes suggesting that vitamin D replacement might be a potential preventive measure for incipient diabetes. Vitamin D deficiency is highly prevalent (18-50%) among the U.S. Hispanic population. If we found a positive association between low vitamin D and high plasma glucose, it could suggest a simple preventive intervention for diabetes in New Mexico.

Prior literature has suggested an association between vitamin D deficiency and prediabetes (29). Our analysis will examine whether such an association exists in a cohort of elderly Hispanics and NHW of New Mexico.

Significance

Diabetes represents a significant impact on quality of life and our national healthcare economy. Therefore, by identifying whether there is an association between vitamin D deficiency and prediabetes as measured by fasting plasma glucose, we can inform and develop a prevention strategy for diabetes. The proposed research has the potential to help prevent the progression of the disease at an early stage, and thus could reduce health care costs attributed to diabetes. Our research study will contribute to an understanding of the association of fasting plasma glucose (FPG) and vitamin D status in a cohort of older Hispanics and NHW of New Mexico. It has been estimated that each year up to 11% of individuals with prediabetes will develop diabetes (30). Nazarian et al. found that the insulin sensitivity improved in subjects with high-dose vitamin D supplementation (10,000 IU) daily for four weeks delaying the progression to diabetes (16). Vitamin D thus may be replenished with a simple inexpensive intervention through improvements in nutrition or by predetermined exposure to sunlight.

Innovation

Even though Hispanics have a higher prevalence rate of vitamin D deficiency, the link between vitamin D deficiency and prediabetes is still unknown as are differences in vitamin D deficiency between Hispanic and non-Hispanic white

populations in New Mexico.   Our research study is innovative because it will help to identify whether vitamin D deficiency is linked to prediabetes, which may ultimately lead to a low risk intervention for the prevention of diabetes.   New Mexico is the ideal site for the conduct of this research because of the multi-ethnic population (Hispanic, non-Hispanic white, American Indian) and the unique environment of low latitude and high altitude where UV exposure is high. Our focus on Hispanic and non-Hispanic whites of New Mexico adds strength because we can differentiate vitamin D deficiency in both groups and New Mexico has a high proportion of Hispanic population which may increase the risk for high prevalence of vitamin D deficiency and diabetes (3, 13).   Our approach would allow us to identify the association of vitamin D deficiency and prediabetes in a highly vulnerable population with diabetes and skin types with different degrees of melanin content (which can lead to reduced vitamin D synthesis), where simple interventions are urgently needed to prevent the disease. We utilized data from the New Mexico Elder Health Survey (NMEHS), where subjects were randomly selected from the Medicare rolls of Bernalillo County, Albuquerque.

CHAPTER 2: MATERIALS AND METHODS

The data for this research study were extracted from New Mexico Elder Health Survey (NMEHS) based on a Medicare beneficiary population. The NMEHS is the first population based epidemiological survey to examine health and health related issues of elderly (65 years and older) Hispanic and non-Hispanic white residents in Bernalillo County, Albuquerque, New Mexico (31). Data were collected between May 1993 and September 1995. The survey was composed of a two-tiered design consisting of a standardized home interview and an extensive clinical examination. Initially a total of 2,200 names were randomly selected from a listing of 50,700 individuals 65 years and older that was provided by Health Care Financing Administration (HCFA) which administers the Medicare program (31) (32).

The subjects' information on age, sex and race was included in the HCFA listing. Ethnicity was assigned based on surname pattern by GUESS (Generally Useful Ethnic Search System), a computerized program. Ethnic heritage was confirmed by self-report from participating subjects. Of these 2,200 subjects, 534 had died, moved, could not be contacted or were otherwise found to be ineligible. Of the remaining eligible subjects, 1,130 (67.8 percent) completed the home interview and 883 (53 percent) (Fig 5) completed the full physical examination and formed the basis of our sample. Two samples of blood were drawn at the time of physical exam where the fasting plasma glucose was measured and vials of

centrifuged sera samples were stored in a freezer in the Molecular Epidemiology

laboratory at the University Of New Mexico (UNM) for future

New Mexico Elder Health Survey

Fig 5

INITIAL SAMPLE SELECTION
n = 2,200

Eligible for Survey
n = 1,666

Refusals
n = 536

Eliminated from sample
•Errors in database, n = 315

Participated in Home Interview
n = 1,130

Interviewed but did not meet ethnicity criteria
n = 29

•Unable to locate address, n = 78

•Unable to establish contact, n = 133

Eligible for Full Examination
n = 1,101

•Did not meet ethnicity criteria, n = 8

Refused
n = 218

Completed Full Exam
n = 883

102 Missing Key Variables

781 Complete Records

200 Randomly Selected for Analysis

use. Other information such as body mass index (BMI) was also measured

during physical examination.   All subjects gave informed consent, and all

procedures approved by Human Research Review Committee of the UNM,

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School of Medicine (31). All data in this study were de-identified and thus exempt from human subjects' considerations.

Fasting Plasma Glucose

Fasting plasma glucose (FPG) is categorized according to American Diabetes Association (ADA) (Fig 6) as follows:

Fig 6 ADA Classification of FPG
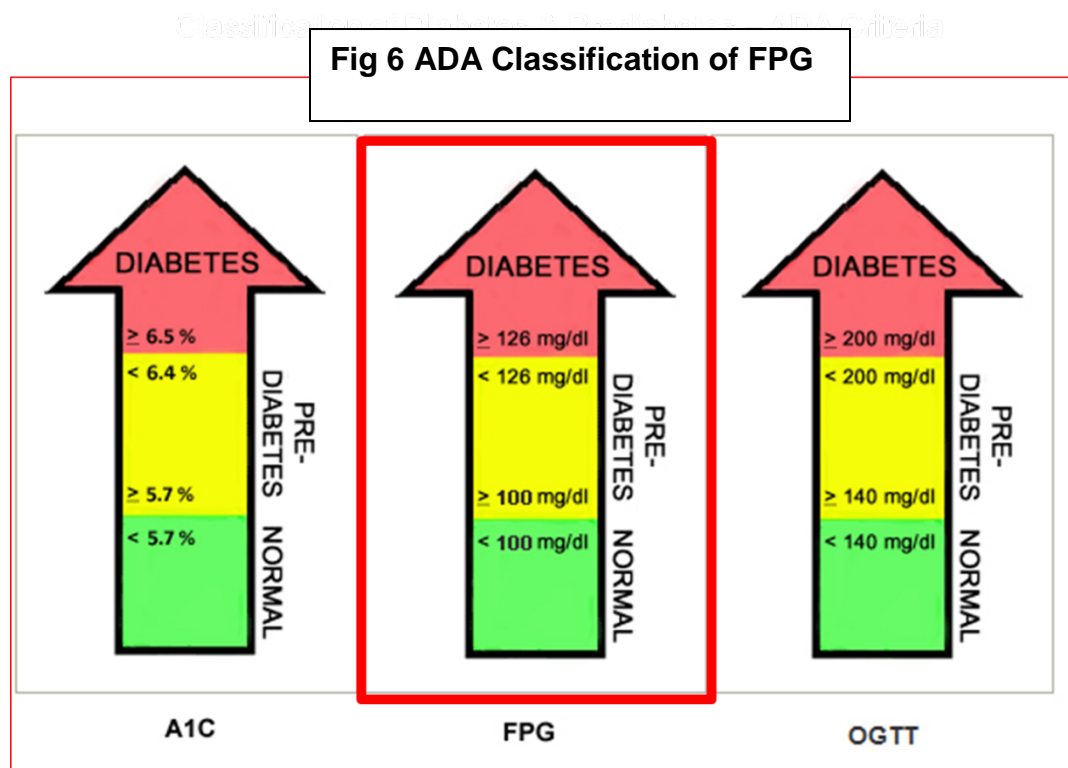
The ADA criterion uses hemoglobin A1c level for diagnosis of diabetes which is a measure of average plasma glucose over the 2-3 month period. Since we were presented only with FPG level, we grouped our study subjects into two categories normal and prediabetes based on FPG. When FPG is less than 100 mg/dL it is considered as normal; between 100 mg/dL and 125 mg/dL is considered as prediabetes.

Vitamin D

The lifespan of vitamin D levels in sera are substantially stable for well over 20 years (personal reference Dr. Ronald Hurst, Heartland Assays, Ames, Iowa) when stored at -80° celsius. We therefore utilized the stored vials of frozen sera containing at least a minimum of 125 µL. The sera samples were placed in dry ice for maintaining the integrity of the samples and were shipped out to Heartland Assays, Ames, Iowa, one of the reputed laboratories for vitamin D measurement in the nation. Vitamin D levels were measured using chemi-luminescent immunoassay and the levels were expressed in ng/mL.

Vitamin D levels were initially categorized into 3 groups (Table 1) according to Institute of Medicine (IOM) criteria: Normal (20 ng/mL and above), Inadequate (12 ng/mL to 20 ng/mL) and Deficient (less than or equal to 12 ng/mL).

Table 1 Institute of Medicine Classification of Vitamin D Levels

Adequate	Inadequate	Deficient
≥20 ng/mL	12-20 ng/mL	≤12 ng/ml

Because the number of vitamin D belonging to deficient strata was very small (n=28), we combined inadequate with deficient group for a better power to the study. Therefore, in lieu of 3 categories of vitamin D, we analyzed into 2 categories: normal (≥20 ng/mL) and deficient (<20 ng/mL) vitamin D groups.

BMI

The study subjects were categorized according to CDC classification into 3 groups (Table 2): Normal BMI (18.5-24.9); Overweight BMI (25.0-29.9) and Obese BMI (≥30).

Table 2 CDC† Classification of BMI	
BMI*	Weight Status
<18.5	Underweight
18.5-24.9	Normal
25.0-29.9	Overweight
≥30	Obese

† Center for Disease Control and Prevention
*Body Mass Index

Age, Sex and Ethnicity

The study subjects are composed of both males and female participants with distribution of Hispanics and non-Hispanic whites of New Mexico (Table 3). Since the subjects were obtained from NMEHS containing only Medicare recipients from Bernalillo County, Albuquerque, NM, every participant is 65 years and older. We categorized age as follows:

Table 3 -- Age distribution of ethnicity and gender of 200 study subjects					
Category	**Age range**	**NHW**	**Hisp**	**Males**	**Females**
1	65-70 years	26	28	27	27
2	70-80 years	66	50	62	54
3	>80 years	18	12	14	16

Study Design

This is a cross-sectional study design. This study serves as a useful platform to examine the association of vitamin D in prediabetes population. This is a cross-sectional study design. For the present study, we excluded 102 subjects out of 883 participants because of missing data on key variables such as BMI and glucose.   This resulted in a total of 781 subjects (883-102). Due to the limited available funding, we analyzed only 200 subjects (Fig 5) of the total 781 people. We randomly selected 200 out of 799 subjects using STATA software program to analyze the association of vitamin D deficiency and prediabetes. Vitamin D levels were measured on these selected 200 subjects whose serum samples were stored in the Molecular Epidemiology Laboratory, University of New Mexico, Albuquerque, NM.   Aliquots of these serum samples were analyzed by Heartland Assay, Ames, Iowa, that specializes in vitamin D measurement.

For our study we wanted to analyze the association of vitamin D deficiency with normal and prediabetes population. Our variables included in the study are:

fasting plasma glucose (FPG), vitamin D, body mass index (BMI), age, sex and ethnicity. To do this, we conducted the following specific aims:

Aim 1: Determine the association between vitamin D deficiency and prediabetes among elderly New Mexicans from a subset of NMEHS cohort.

Introduction for Aim 1: The objective of this research was to elucidate the association between vitamin D deficiency and prediabetes, among the Hispanic and non-Hispanic white New Mexican elderly population in the NMEHS. A number of studies suggest an association exists between the vitamin D deficiency and diabetes but no direct relationship has been established. Moreover, little is known about the link between vitamin D and prediabetes. Our study would thus allow us to determine whether vitamin D deficiency has an association with prediabetes among the representing group of Hispanic and non-Hispanic whites 65 years and older.

Data Analysis for Aim 1: Utilizing STATA, we performed univariate analysis including frequency distributions; Pearson chi-square test to find association for categorical variables; t-test to find mean values of continuous variables; simple linear regression to find association between continuous variables; and multivariable logistic regression analysis for categorical variables with prediabetes as outcome to assess the odds ratios and the impact of multiple variables including vitamin D level, sex, BMI, age and ethnicity.

Aim 2: Determine whether the association between vitamin D deficiency and prediabetes is stronger among Hispanic than non-Hispanic whites from NMEHS sample.

Introduction for Aim 2: The prevalence of diabetes is known to be higher among Hispanics than non-Hispanics as is the vitamin D deficiency (2, 3, 4). However, since Hispanics have different skin types than non-Hispanic whites (14, 25), attributable to melanin contents and melanin acts as a sunscreen reducing the synthesis of vitamin D from ultraviolet B rays, we expected to find a stronger association of vitamin D deficiency and prediabetes among Hispanics than non-Hispanic whites in the NMEHS sample.

Data Analysis for Aim 2:

We used linear regression models to assess the association between continuous variables; multivariable logistic regression analysis for vitamin D deficiency (predictor) on prediabetes (outcome) by interaction model where BMI, gender, age and ethnicity are covariates.

Sample Size and Power Analysis for Aim1: We used SAS Power Analysis to calculate power of the study (Table 4). At a significance level of 0.05, a sample size of 200 observations achieves 81% power to detect an OR of 2.25, when the risk factor proportion is 50% and disease prevalence is 50%.

Table 4   Power Analysis

Number of subjects	Significance Level	Odds Ratio	Power
200	0.05	1.5	0.3
200	0.05	1.75	0.5
200	0.05	2.00	0.68
200	0.05	2.25	0.81
200	0.05	2.5	0.89

Statistical Analyses

The data analyses were performed by using STATA software program (Stata Corp, 4905 Lakeway Dr, College Station, TX 77845) and SAS 9.3 for windows (SAS Institute Inc. Cary, NC).

Inclusion and Exclusion Criteria

Our population included Hispanics and non-Hispanic whites of 65 years and older containing both males and females. Our study also contained subjects with normal (≥20 ng/mL) and deficient (<20 ng/mL) levels of vitamin D (Table 5). We excluded participants with hypoglycemic levels of glucose (FPG<60 mg/dL), diabetes (FPG≥126mg/dL) and underweight BMI (<18.5).

Table 5   Criteria for Study Subjects

Inclusion Criteria	Exclusion Criteria
• 65 years and older	• Missing values
• Hispanics and NHW	• BMI – underweight
• Glucose – Normal and Prediabetes	• Hypoglycemia and Diabetes
• Vitamin D – Normal and Deficient	

CHAPTER 3: AIM 1 RESULTS

The first aim of the study was to determine whether an association between vitamin D deficiency and prediabetes exists. We hypothesized that vitamin D deficiency is associated with increased odds for having prediabetes. To analyze the data for Aim 1, we utilized STATA and SAS software program. We conducted descriptive analysis to study the characteristics of the population, Pearson Chi-square test and linear regression analysis to measure association between categorical and continuous variables; and multivariable regression analysis for prediabetes as outcome measure.

Descriptive Analysis:

Study Population:

Our study subjects contained slightly higher percentage (55%) of non-Hispanic whites (NHW) than Hispanics (45%) (Table 6). The participants were relatively equally distributed among males (n=103) and females (n=97). The distribution of prediabetes was slightly higher than 50% of the study population (n=103) and normal glucose category was close to 50% of population (n=97). Within the 3 categories of BMI status, 38% belonged to normal weight, 44% were overweight and 18% were obese. The distribution of vitamin D levels between normal (n=101) and deficient (n=99) status were relatively equal.

Table 6   Study Population Characteristics

	NHW		Hispanics	
	Males	**Females**	**Males**	**Females**
Glucose Status				
Normal	27	27	18	25
Prediabetes	34	22	24	23
BMI Status				
Normal	26	17	14	19
Overweight	25	21	22	20
Obese	10	11	6	9
Vitamin D Status				
Normal	44	21	27	9
Deficient	17	28	15	39
Sex	61	49	42	48

Table 7 Univariate Association of Continuous Predictor Variables with Prediabetes

	Normal Glucose	Prediabetes	
Variable	**Mean ± SD**	**Mean ± SD**	**p-value**
Serum Vitamin D (ng/mL)	20.43 ± 8.32	20.99 ± 11.17	0.69
BMI (kg/m²)	25.36 ± 3.37	27.76 ± 4.18	<0.001
Age (years)	74.56 ± 5.72	73.49 ± 5.42	0.18

We analyzed the mean values of potential continuous predictors among the two FPG statuses (Table 7). There was no significant difference in mean vitamin D level between normal and prediabetes FPG. The average vitamin D values were similar among normal glucose and prediabetic subjects (p-value=0.69). The mean age distribution among these two FPG statuses also was similar and non-significant (p-value=0.18). Prediabetics had a statistically significant higher mean

BMI as compared to normal glucose subjects with lower mean BMI (p-

value=0.0001) though both BMI belonged to overweight category.

We then wanted to determine the measure of association, on explanatory

categorical variables, including vitamin D status and normal versus prediabetes

as determined by FPG by Pearson Chi-square test, a simple univariate analysis

(Table 8).

	Normal Glucose	Prediabetes	Total Number	Chi-sq p-value
Table 8 Association of Categorical Variables with FPG Status among 200 Randomly selected subjects with Vitamin D quantified				
BMI				<0.0001
Normal Weight	62%	38%	76	
Overweight	50%	50%	88	
Obese	17%	83%	36	
Vitamin D (ng/mL)				0.398
Normal	46%	54%	101	
Deficient	52%	48%	99	
Age (years)				0.332
60-70	41%	59%	54	
70-80	50%	50%	116	
≥80	57%	43%	30	
Ethnicity				0.853
NHW	49%	51%	110	
Hisp	48%	52%	90	
Sex				0.161
Males	44%	56%	103	
Females	54%	46%	97	

The distribution of vitamin D levels among normal and prediabetes groups were

not significantly different.   We did find a statistically significant difference with

BMI distribution between the prediabetes and normal glucose groups (p-value<0.0001), where 83% were obese and prediabetic as compared to 17% obese with normal glucose. Overall, the distribution of other covariates, age, ethnicity and gender did not have significant association with glucose levels. To analyze the relationship between two continuous variables, vitamin D levels and fasting plasma glucose, we constructed a simple regression analysis (Fig 7) with and without the vitamin D outlier value (106.6 ng/mL).

Fig 7   Regression Plot – FPG and Serum Vitamin D Level

Slope= -0.03
p-value=0.74
R^2 = 0.0006
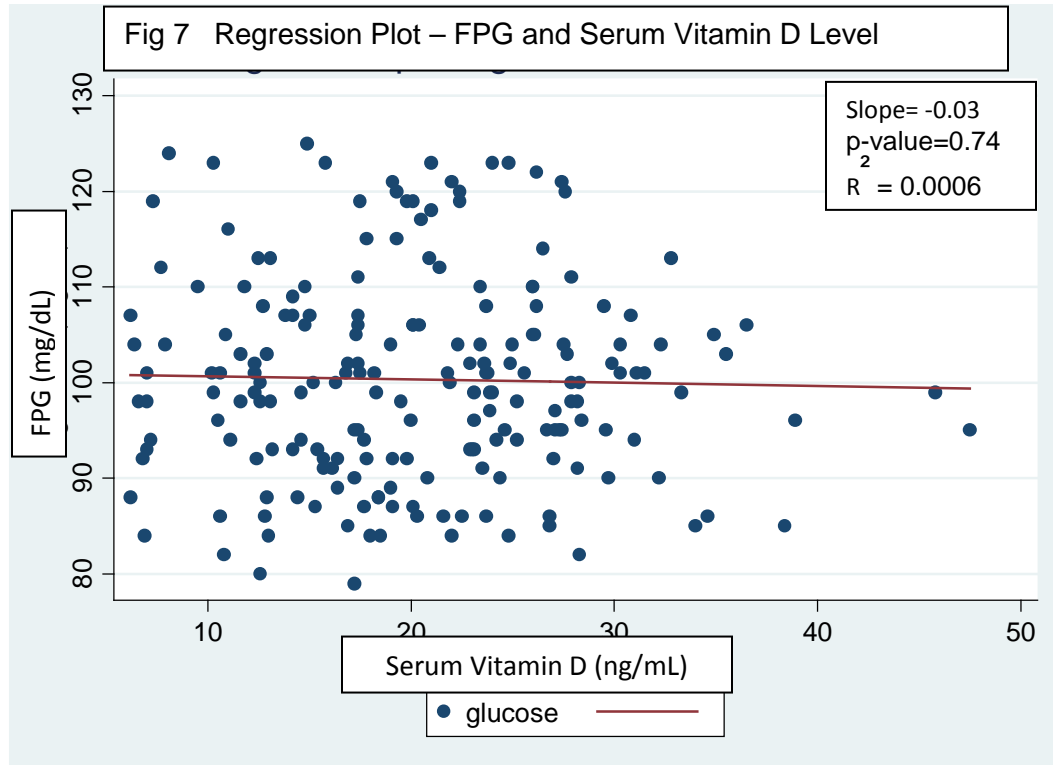
The relationship between vitamin D excluding the outlier and FPG is displayed in Figure 8, where regression line indicates a non-statistically significant slightly negative relationship between the two variables (excluding outlier: slope = -0.03,

p=0.74). When the outlier was included we saw a slightly positive relationship not significant (slope=0.08, p=0.30). Vitamin D levels accounted for approximately 0.06% of the variation in FPG (R²=0.0006). We included the outlier value in the calculation wherever we analyzed vitamin D data as categorical variable.

As previously described, we found that the distribution of BMI was significantly different between FPG status groups, so to further explore this relationship we performed a simple linear regression analysis between the two continuous variables, BMI and FPG with latter as the outcome (Fig 8). There was a positive relationship between the two variables (p-value<.001) where BMI accounted for 8% of the variation in glucose (R²=0.08).

Fig 8   Regression Plot – FPG with BMI

Slope = 0.78
p-value<.001
$R^2 = 0.08$
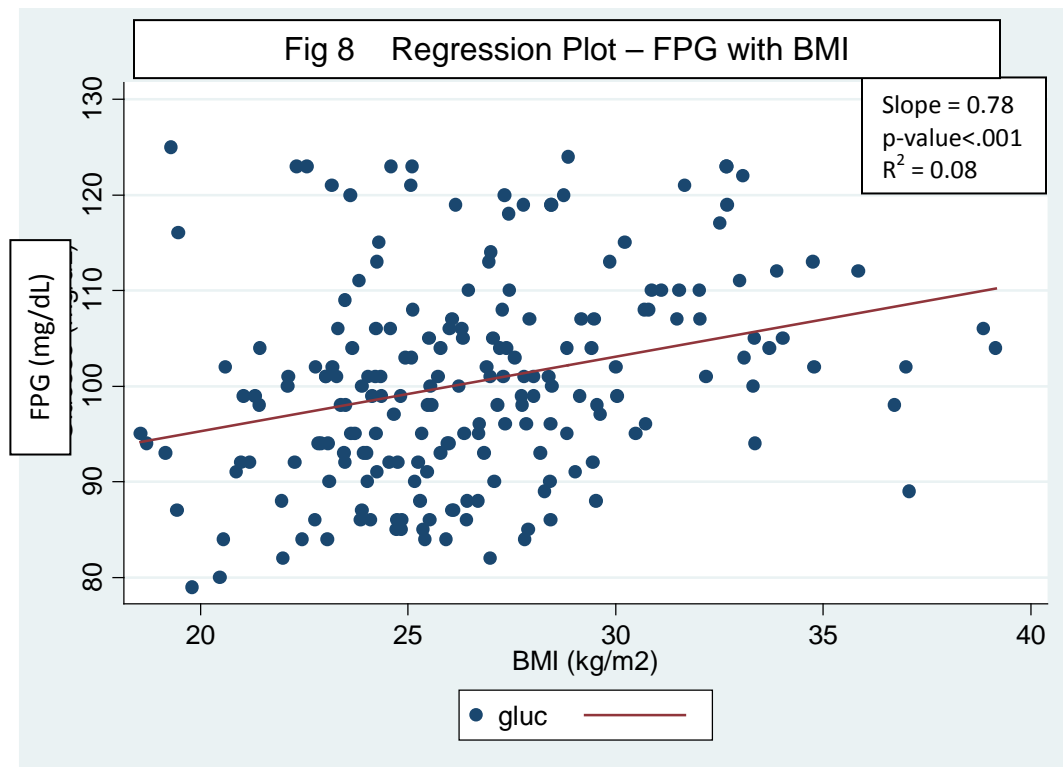
Multivariable Logistic Regression Analysis

To further explore the relationship between vitamin D and prediabetes, we conducted a multivariable logistic regression analysis (Table 9) to adjust vitamin D for other significant covariates.

Table 9   Multivariable Analysis – Prediabetes Outcome

Predictor Variables	Odds Ratio	95% CI
Vitamin D deficiency	0.88	(0.46, 1.69)
Hispanic Ethnicity	1.17	(0.64, 2.14)
BMI-Overweight	1.48	(0.78, 2.80)
BMI-Obese	8.06	(2.93, 22.20)

Additional covariates in the model are age & sex

From this analysis, we were unable to reject the null hypothesis for Aim 1 because we did not find an association between vitamin D deficiency and prediabetes, OR=0.88, (CI .46, 1.69). This analysis found no increased odds for having prediabetes in Hispanics; however the role ethnicity plays in prediabetes is further discussed in the next chapter. BMI status had a significant role for having prediabetes. In general our subjects who were obese had significantly higher odds, OR=8.06, CI (2.93, 22.20) for having prediabetes than normal weight subjects, whereas, overweight subjects were 1.48 times likely to develop prediabetes than normal weight people, though not significantly associated.

CHAPTER 4: AIM 2 RESULTS

The second aim of the study was to determine whether the degree of association between vitamin D deficiency and prediabetes was stronger among Hispanics than NHW.   We hypothesized that the association would be stronger among Hispanics than NHW elderly New Mexicans.

We analyzed the mean values of potential continuous predictors among the two categories of ethnicities (Table 10).

**Table 10   Association of Continuous Variables among Ethnic Groups**

	NHW	Hispanics	p-value
	Mean ± SD	Mean ± SD	
Serum Vitamin D (ng/mL)	22.3 ± 11.0	18.8 ± 8.0	0.01
Fasting Plasma Glucose (mg/dL)	99.4 ± 9.6	101.6 ± 12.0	0.15
BMI (kg/m²)	26.7 ± 4.2	26.4 ± 3.7	0.61
Age (years)	74.4 ± 5.3	73.6 ± 5.8	0.30

The mean values of potential predictors among the two ethnic groups are listed as above (Table 10). Hispanic subjects had a statistically significant lower mean vitamin D level (18.8 ng/mL) which falls in the deficient range (p-value=0.01) as compared to NHW with mean vitamin D level (22.3 ng/mL) in the normal range. Previously Hispanics have been shown to have lower vitamin D level than NHW (16). The mean values of fasting plasma glucose (FPG) for Hispanics was slightly higher, but not statistically different than NHW (p-value=0.15).   Both ethnic groups' mean value of BMI status were similar and in the overweight

category. Similarly the mean age (74 years) was equal among Hispanics and NHW subjects.

We wanted to determine how the FPG was distributed among the two ethnic groups based on their vitamin D status. Therefore we constructed box plots for prediabetes as outcome variable with vitamin D and ethnicity as categorical explanatory variables (Fig 9).

Fig 9   FPG Distribution among NHW & Hispanics Stratified by Vitamin D status
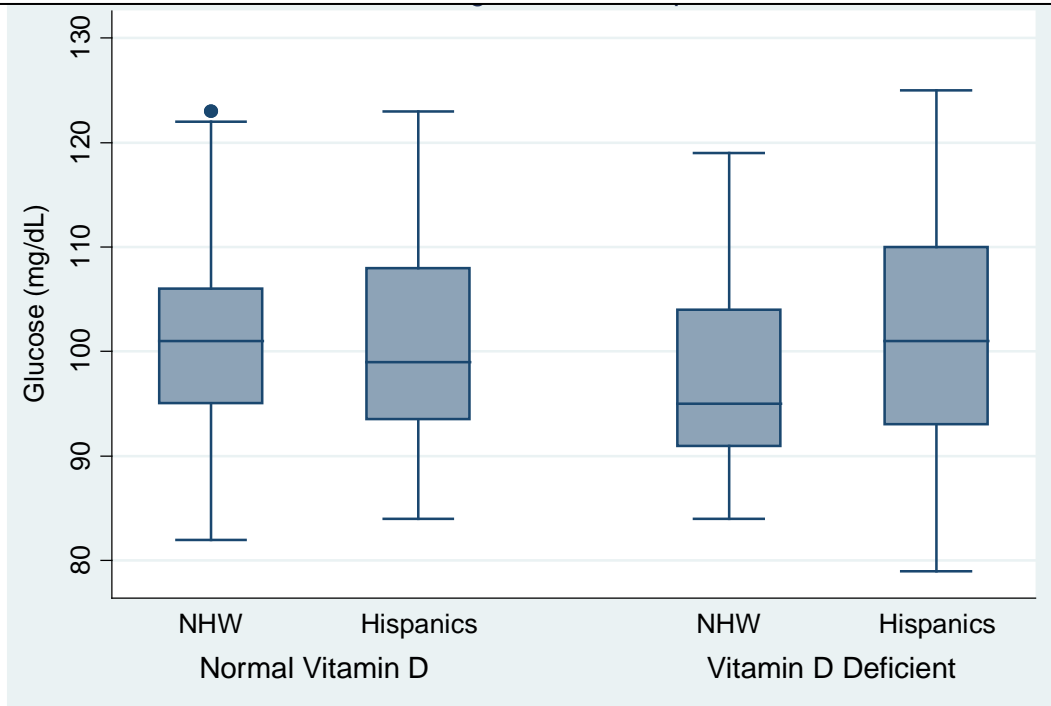
When our sample was stratified by vitamin D levels, Hispanic and NHW participants did not significantly differ in terms of BMI or age (Table 10). On the other hand, among vitamin D deficient participants, the median glucose for Hispanics with vitamin D deficiency was higher (102 mg/dL) than NHW (94 mg/dL) although not significant. However, the boxplot in Figure 10 indicated that there may be an interaction between ethnicity and vitamin D levels and therefore

we performed a multivariable logistic regression analysis including an interaction

term to adjust vitamin D for other significant covariates (Table 11).

Table 11 Multivariable Regression Analysis by Interaction – Prediabetes as Outcome		
Predictor Variable	**Odds Ratio**	**95% CI**
Hispanics with Vitamin D Deficiency vs NHW with Vitamin D Deficiency	2.42	(1.00, 5.85)
Overweight vs Normal Weight	1.62	(0.84, 3.11)
Obese vs Normal Weight	9.17	(3.23, 26.03)
Age: 70-80 vs ≥65-70 years ≥80 vs ≥65-70 years	0.67 0.64	(0.33, 1.37) (0.24, 1.69)
Female vs Male	0.57	(0.30, 1.10)

From the above analysis we found that Hispanics having vitamin D deficient

status is associated with 2.4 fold increased odds for prediabetes as compared to

NHW (p=0.02). Therefore, we reject the null hypothesis for Aim 2.

There was also significant finding among obese population more likely to have

prediabetes, OR=9.17, CI (3.23, 26.03) than normal weight people.

Table 12   Association of Vitamin D Levels with Age Category

Age category	Normal Vitamin D	Deficient Vitamin D	Chi-sq p-value
65-70 years	30	24	=0.232
70-80 years	60	56	
≥80 years	11	19	

We wanted to evaluate whether age has an association with vitamin D level as described by previous studies (18, 20). But we did not find a correlation among increasing age and vitamin D deficiency (p-value=0.232) (Table 12).

CHAPTER 5: DISCUSSION AND CONCLUSION

We aimed to determine whether there is an association between vitamin D deficiency and prediabetes among New Mexico Elder Health Survey (NMEHS) subjects. Our study found no association between vitamin D deficiency and prediabetes opposite to our hypothesis for Aim 1. However, we did find that our elderly New Mexican Hispanic subjects with vitamin D deficiency had a 2.4 fold increased risk for pre-diabetes, as we had hypothesized for Aim 2. This finding indicates that there may be a cumulative effect of risk factors influencing the outcome more than the individual factors acting independently. Hispanics were significantly more likely to be vitamin D deficient, and those who were deficient were more likely to be prediabetic.

One reason for the lack of an association between vitamin D deficiency and prediabetes as hypothesized in Aim 1 could be the small sample size (N=200), although our power analysis did indicate that we would have 81% to detect a 2.25-fold increased risk if the risk factor constituted 50% of the sample. The result may be different when the rest of the sample is analyzed (N=581) as a larger sample size would have more power to detect the relationship better between the low vitamin D and prediabetes than a smaller study group.

Hispanic subjects' mean vitamin D level compared to NHW was significantly lower and this may be due to predisposing factors such as darker skin types with some genetic factor with the Hispanic ethnicity itself leading to the deficiency.

This finding is in agreement with previous research work by Chen et al. (14) and Forrest et al. (18).

Obesity was another risk factor that appeared to play a significant role in prediabetes independently.   According to the 2009-2010 National Health and Nutrition Examination Survey (NHANES), 35.7% of American adults were obese (26). Nevertheless, our elderly population's obesity rate in 1993-1995 was half the prevalence rate (18%) compared to the 2009-2010 national data.

Age as was described in previous studies by MacLaughlin et al. and Forrest et al. as a risk factor for lower serum vitamin D level. But in our study, age was not associated with low serum vitamin D. However, as all subjects were 65 years or older, the age range was possibly too narrow to see a difference.   It may also be possible that the older age groups used daily vitamin D supplements or vitamin D fortified food that helped to maintain their levels. Our analysis on increasing age and its association with vitamin D deficiency did not correlate to earlier study findings as suggested by MacLaughlin where increasing age caused a decrease in DHC content in the stratum basale that led to lower vitamin D synthesis (20).). But we did not see such a trend consistent with age (Table 12). Also another study by Forrest et al. found that older adults were more vitamin D deficient than younger adults (18).

Limitations

This is a cross-sectional study design; therefore, the data are only valid for associations and not causal effects.   Our study was limited to the evaluation of associations of vitamin D deficiency and prediabetes with elderly subjects of 65

years and older.   It is not certain whether the results of our study can be applied to a younger population. In addition, as we only studied Hispanics and NHW in New Mexico generalizability to other races such as African Americans or Asians or other Hispanic sub-groups is limited. Since New Mexico gets sunlight most of the year, comparison of our study to similar subjects located at different latitudes may not be applicable where there is limited sunshine at lower elevation.

Our study is a preliminary analysis of small sample size (N=200). To obtain a more definitive evaluation of our hypotheses, we need to examine the complete sample size of 581 subjects. Our power analysis demonstrated that we could detect an odds ratio of 2.25 between the prediabetes and normal subjects and the odds ratio we found was 2.42, so that we may have missed associations with lower estimates of effect.

There was missing information from our data set that possibly could restrict our findings. For example, residual confounding could take place by unmeasured variables such as hemoglobin A1c values, sun exposure, vitamin D intake, lactose intolerance status and whether subjects were on medications that would impair vitamin D metabolism such as steroids, cholestyramine, phenobarbital or phenytoin.   Also, the duration of prediabetes or vitamin D deficiency in this population is unknown, and this might determine the severity of the disease.

Strengths

Because ours is a cross-sectional study where the data had already been carefully collected, it was less expensive to conduct and quicker to analyze than

a longitudinal cohort study.   There are reasons why our findings may be generalizable with external validity. Because we selected 200 subjects randomly from a community based population, we have less chance for selection bias and therefore greater external validity. Our study can be generalized to other elderly Hispanic and NHW population of New Mexico and to other people from similar backgrounds. The findings of our study indicate that one might prevent the progression of prediabetes to diabetes with simple vitamin D supplements when deficient.   In addition, obesity, as a known risk factor for diabetes may also be involved at an earlier stage of prediabetes (3). Therefore, preventing prediabetes by vitamin D supplementation along with maintaining a healthy weight help delay diabetes, maintain quality of life and reduce health care costs.

Future Directions

There is a great public health need to learn more about vitamin D deficiency and its role in preventing diabetes.   Our preliminary findings helped us understand the positive relationship between vitamin D deficiency and prediabetes. Therefore, it is important to analyze the rest of the sample population (N=581) so that these results might be stronger and usefully translated to the general population.   We will include the participants' usage of medications and other supplements in the analysis. We will also consider an age and skin type (Fitzpatrick scale) interaction in our full analysis of larger sample size. We plan to use our Clinical and Translational Science Center (CTSC) grant to conduct the rest of the analysis for more definitive answers.   We will publish our research findings in a manuscript once the analysis is complete.

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