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Disparity of Vitamin D Deficiency between Mexican-Americans and Non-Hispanic Whites with Type 2 Diabetes Mellitus, NHANES 2001-2006

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Disparity of Vitamin D Deficiency between Mexican-Americans and Non-Hispanic Whites with Type 2 Diabetes Mellitus, NHANES 2001-2006

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Abstract

Disparity of Vitamin D Deficiency between Mexican-Americans and Non-Hispanic Whites with Type 2 Diabetes Mellitus, NHANES 2001-2006

By Janet Figueroa

Context Vitamin D deficiency is associated with cardio-metabolic and diabetes-related complications in those with type 2 diabetes mellitus (T2DM). This deficiency is also much more prevalent in Mexican-Americans compared to non-Hispanic Whites. Information about the causes of the observed vitamin D deficiency disparity in subjects with T2DM has not been reported using NHANES' assay-adjusted serum 25(OH)D measurements.

Objectives The objective of this study was to estimate the disparity of vitamin D deficiency, measured with serum 25(OH)D levels, between Mexican-Americans and non-Hispanic Whites with type 2 diabetes mellitus.

Design, Setting, and Participants Data was obtained from NHANES, a stratified, multi-stage and nationally representative survey conducted across the US on the non-institutionalized population. Analysis was restricted to adults aged 18-85, who were of Mexican-American and non-Hispanic White ethnicity (n=292, 483; total n=775), and were diagnosed with T2DM. T2DM was defined by age of diagnoses (>30 years of age) or if currently taking hypoglycemics. All statistical analyses were conducted to take into account the weighting for the multi-stage, combined survey cycles.

Outcome The outcome of interest was vitamin D deficiency, defined as serum 25(OH)D levels <15 ng/mL and any disparity in deficiency between Mexican-Americans and non-Hispanic whites with T2DM.

Results In those with T2DM, vitamin D deficiency was much more prevalent in Mexican-Americans compared to non-Hispanic whites, a disparity also seen in the crude logistic regression model (38.7% vs. 20.0%, $p<0.0001$; OR 2.51, 95% CI 1.66-3.79). Adjusting for potential confounders, such as supplement use, demographics, behavioral risk factors, cardiovascular-related risk factors (41% attenuation), and diabetes medication type (46% attenuation), resulted in attenuation of the crude OR, with the latter two, resulting in the greatest attenuations.

Conclusion The observed vitamin D deficiency disparity between Mexican-Americans and non-Hispanic whites with T2DM was greatly attenuated by potential confounders, highlighting further disparities in the occurrence of the risk factors that inflate the true deficiency. Future studies should explore the nature of these observed confounders and how to address them in order to reduce the overall risk of vitamin D deficiency, which can complicate skeletal as well as non-skeletal effects in those with T2DM.

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TABLE OF CONTENTS

Background/Introduction…….....1

Statistical Methods…….…….9

Results…….…..15

Discussion…….17

Conclusion…….21

References…….24

Tables…….29

Summary & Public Health Implications……33

Background/Introduction

The prevalence of type 2 diabetes mellitus is on the rise and one of the most widely diagnosed chronic diseases (Badenhoop, 2015). There are more than 26 million Americans with diabetes mellitus and an additional 80 million with 'prediabetes' (elevated HgA1c), all prone to increased morbidity and mortality with type 2 diabetes mellitus accounting for 90-95% of US cases (Cangoz et al, 2013).

Since the early eighties, diabetes prevalence among Mexican-American adults has increased faster in comparison to other ethnic groups (eg. Non-Hispanic whites). The age-adjusted prevalence of type 2 diabetes mellitus in Hispanic-Americans is twice as large as it is in white Americans. By 2020, the percentage of Hispanics in this country with diagnosed diabetes will increase by 107%, compared to an estimated increase of 27% for white Americans (Freeman, 2010). Several explanations for this increasing disparity have to do with overall increases in obesity prevalence, poorer lifestyle behaviors in Hispanics, lower diabetes self-management, and the fact that Hispanic Americans are less likely to receive preventive healthcare compared to non-Hispanic whites (Harris et al, 2010). Previous cross-sectional studies have shown a relationship between diabetes (including cardiovascular complications) and vitamin D levels in ethnic minorities (Penckofer et al, 2008). Minority groups, in particular Blacks and Mexican-Americans, have higher risks of vitamin D deficiency compared to Whites (Gupta et al, 2012). Castro et al. state that the highest prevalence of low vitamin D levels can be found in the Hispanic population (Castro et al. 2014). Some factors that place Hispanics at a greater risk for low vitamin D levels include obesity, darker skin pigmentation, latitude of residence (less sunshine), and presence of renal insufficiencies (Castro et al, 2014).

Vitamin D, Diabetes, and Supplementation

Vitamin D is a fat soluble steroid that requires an ultraviolet B dependent conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) (Baz-Hecht et al 2010, Chaoxun 2013). The vitamin D_3 is hydroxylated in the liver by a hydroxylase to 25-hydroxycholecalciferol (25(OH)D), then hydroxylated again in the kidney to the biologically active steroid hormone $1,25(OH)_2D$ (Baz-Hecht et al 2010 & Gupta et al 2012). Since the main circulating vitamin D precursor with the longest half-life is 25(OH)D, measuring its concentration is the most adequate form of evaluating vitamin D levels (Judd et al, 2009). Serum 25(OH)D is considered the best biomarker for measuring dietary and endogenous vitamin D sources (Yetley et al, 2001).

Aside from being a necessary component in calcium metabolism and absorption, vitamin D has been linked to other processes, including a role as a risk factor for diabetes complications and other cardiovascular risk factors. An explanation for this may lie in the ubiquitous nature of vitamin D receptors, found in pancreatic beta cells, immune system, muscle, and heart cells (Castro et al, 2014). Vitamin D has both genomic and non-genomic pathways, direct and indirect pathways affecting insulin secretion, beta-cell function, and insulin resistance (Bajaj et al, 2014; Saedisomeolia et al, 2014). Researchers have also shown that vitamin D deficiency is much more prevalent in diabetics compared to non-diabetic patients (Bajaj et al, 2014). One proposed mechanism for this involves megalin receptors, endocytic receptors that facilitate 25(OH)D entry into target cells for activation and use in the body (Castro et al, 2014). Calcium levels, which need vitamin D to have calcium absorbed, are linked to insulin release and transport of glucose into cells (Al-Daghri et al, 2012; Castro et al, 2014). Vitamin D is also linked to cardiometabolic risk factors including elevated blood pressure and lipid levels, the latter through a proposed regulatory action that increases the activity of lipoproten lipase in adiposity (Saedisomeolia et al,

2014). One study found serum 25(OH)D inversely associated with systolic blood pressure and overall hypertension (Ganji et al, 2011). Vitamin D plays a role in regulating blood pressure through the renin-angiotensin-aldosterone system (RAS). RAS regulates blood pressure and in obese persons with low vitamin D levels, RAS is unregulated, leading to elevated blood pressure (Ganji et al, 2011). Unregulated RAS in obese persons may be due to vitamin D being sequestered in adipose tissue where all RAS components are synthesized.

The Institute of Medicine recommends a daily vitamin D intake of 4000 IU/day and individual serum concentrations to be around 50 nmol/L (20 ng/mL). NHANES assay-adjusted data from 2005-2006 shows the mean individual 25(OH)D to be around 49. 8 nmol/L, with the highest vitamin D levels coming from sunny regions like California, and high deficiencies in black women and their newborns (Van Schoor et al, 2011). About 30-50% of adults worldwide are vitamin D deficient, with older adults much more vulnerable from having a decreased capacity to make vitamin D from sunlight (Parker et al, 2010). Clinical manifestations of vitamin D deficiencies include muscle weakness, bone pain, fractures, and in children, bone deformations (Van Schoor et al, 2011). However, many vitamin D recommendations only consider maintaining bone health and do not consider the extra-skeletal functions of vitamin D.

The role of supplementation on overall serum 25(OH)D levels

Many studies have shown that lower vitamin D concentrations are associated with increased risk for diabetes and metabolic syndrome (Penckofer et al, 2008), even after adjusting for age, sex, smoking status, season, and body mass index (Chaoxun 2013). Researchers supplemented subjects with vitamin D for increasing vitamin D serum blood levels and later looked at the effect on subsequent diabetes complications. In a retrospective study with vitamin D supplementation given to diabetes patients, baseline levels were at 41.8 nmol/L(±15.0

nmol/L); three months after supplementation, levels peaked at 60.7 nmol/L (±27.7 nmol/L). Obesity prevalence and vitamin D deficiency in the study overall were higher at baseline (Nwuosu et al, 2014). There was a significant reduction in BMI, alanine transaminase, and a clinical decrease in glycohemoglobin (2014), all indicators of type 2 diabetes management. A prospective interventional study also found an increase in mean serum vitamin D levels from baseline to 18 months (32.3 ± 1.5 nmol/L to 54.7±1.5 nmol/L; $p<0.001$) after supplementation, followed by a decrease in LDL and total cholesterol. (Al-Daghri et al, 2012). Logistic regression, adjusting for socio-demographic traits, season, supplement use, physical activity, and insulin resistance, found that there was a 37% decrease in metabolic syndrome risk per standard deviation increase in baseline 25(OH)D after a three-year follow-up (Kayaniyil et al, 2014). Observational and clinical trials have overall shown that vitamin D supplementation may be important in preventing type 2 diabetes and improving lipid/metabolic profiles, brought upon by the overall increase in serum vitamin D levels and its link to extra-skeletal processes (Suzuki et al, 2006; Nwuosu et al, 2014; Al-Daghri et al, 2012).

Lack of Association between Vitamin D and Diabetes-related Complications

A recent Mendelian randomization study by Zheng et al. estimated the unconfounded, causal association between 25(OH)D concentration and risk of type 2 diabetes using data from populations of European descent (Zheng et al, 2014). After looking at four SNPs (single nucleotide polymorphisms) related to 25(OH)D metabolism and synthesis, the study concluded that there was no significant association between low 25(OH)D and risk of type 2 diabetes (Zheng et al, 2014). There were several limitations to this study, however. The four SNPs in the study only account for a small portion (3-6%) of the variation in 25(OH)D concentration and threshold effects of genes on 25(OH)D concentration could also be present. In addition, only data

from populations from European descent populations were studied. Another mendelian randomization study did find that 25(OH)D concentrations were causally associated with reduced blood pressure and hypertension, both cardiometabolic risk factors associated with type 2 diabetes, noting possible limitations from pleitropy from some metabolism variants included in the analyses (Vimaleswaran et al, 2014). Overall, reasons for conflicting evidence may be due to a small number of subjects, vitamin D dosage when administering supplementations as part increasing the overall serum 25(OH)D concentration (comparing baseline and follow-up levels with incidence of metabolic syndrome factors), treatment variations from diabetes medications, and differing duration of studies (Castro et al, 2014; Yiu et al, 2013).

Many studies have been short in overall duration. A study from the Women's Health Initiative Clinical Trials found a lack of association between diabetes type 2 and vitamin D levels (Robinson et al, 2011). Lack of association between vitamin D levels and type 2 diabetes incidence was assessed by measuring serum 25(OH)D concentrations (total body stores of vitamin D), the main exposure of interest (Robinson et al, 2011).

Epidemiology of the Disparity of Vitamin D Deficiency

As stated earlier, vitamin D deficiencies are far more prevalent among minority groups for several reasons. Scragg et al. noted that vitamin D status, along with BMI, were the main determinants of diabetes risk in Mexican-Americans compared to non-Hispanic whites, after adjusting for sex, age, leisure and physical activity (Scragg et al, 2004). This data, however, was not adjusted for possible assay drifts. A look at the NHANES 2005-2006 data revealed that vitamin D deficiency, defined as a serum 25-hydroxyvitamin D concentration less than or equal to 20 ng/mL (50 nmol/L) was highest in blacks, followed by Hispanics, 82.1% and 69.2%, respectively (Forrest et al, 2010). Another cross-sectional study looking at NHANES 1988-1994

also found that serum levels of 25(OH)D below 30 ng/ml (<75 nmol/L) were more prevalent in blacks and Hispanics and in patients with known CVD risk factors, including hypercholesterolemia, obesity, and hypertriglyceridemia (Garrett-Mayer et al, 2012 ; Martins et al, 2007). Race/ethnicity and not income or education has been found to be a strong independent predictor of nutrient concentration and intake in adolescents in a cross-sectional study examining the nutritional and lipid biomarkers (including vitamin D) of US children from data in NHANES 2003-2006 (Kant et al, 2012). One of the aims of this study is to focus on comparing Mexican-Americans, a group vulnerable to vitamin D deficiency, against non-Hispanic Whites, estimating the disparity of vitamin D deficiency among adults with type 2 diabetes mellitus.

Diabetes and cardiovascular risk factors

Cardiovascular-related risk factors, such as hypertension, are also associated with vitamin D levels, a concern for diabetes patients with altered lipid profiles and signs of hypertension. A meta analysis found high levels of serum 25OHD associated with a 43% reduction in cardiometabolic disorders (OR 0.57, 95%CI 0.48-0.68) and 85% of study results showed ORs associating high levels of vitamin D with lower prevalence of cardiometabolic disorders (Parker et al, 2010). However, this meta-analysis may be limited by heterogeneity (eg. differences in study design), and unadjusted confounders. Many studies have found that vitamin D levels are associated with lower prevalence of cardiovascular disease, type 2 diabetes, and metabolic syndrome components (Parker et al, 2010). The severity of coronary artery stenosis was inversely associated with vitamin D levels in 239 coronary angiography patients (Akin et al, 2012), highlighting vitamin D's role in heart health and the importance of noting cardiovascular-related events like atherosclerosis and triglyceride levels when assessing vitamin D status. Ford et al. also looked at quintiles of serum concentrations of vitamin D with waist circumference,

hypertriglyceridemia, low HDL cholesterol, high blood pressure, and hyperglycemia using NHANES III 1988-19994 (2005). Decreasing ORs from a logistic regression analyses, with increasing levels of 25(OH)D were reported (Ford et al, 2005) . However, this study, too, used unadjusted assay data, with possible assay fluctuations in the vitamin D serum measurements biasing results. The correlation of vitamin D deficiency with diabetes and other cardiovascular-related complications underscores the need to address deficiency problems, in particular deficiencies in Hispanic and blacks.

Diabetes Medication and its role in Vitamin D deficiency

Certain diabetic medications may play a role in previously reported associations of vitamin D and diabetes-related complications through various known and unknown mechanisms. Thus, diabetes medication may help explain the previously observed vitamin D deficiency disparities. Mexican-Americans and non-Hispanic whites do differ in the type of diabetes medication they use, with Mexican-Americans mainly using oral medications and not insulin. There are currently no studies that have looked at the potential effect of diabetes medication on the association between vitamin D and cardiometabolic outcomes in the US general population Most studies have simply excluded those taking diabetes medications as part of their study criteria. One case control study looked at the effect of thiazolidenediones (TZD) on vitamin D status among postmenopausal women with type 2 diabetes (Chakreeyarat et al, 2011). Thiazolidinediones are used commonly for treating type 2 diabetes. Results showed that the 25(OH)D levels were higher in TZD users (35.3±1.5 vs 25.9±1.2 ng/dl, $p<0.001$) and that insufficiency was more prevalent in non-TZD users (75.5% vs. 34.6%) (Chakreeyarat et al, 2011). An explanation for this is that PPAR (peroxisome proliferator-activated receptor) is the receptor for TZDs and vitamin D synthesis takes place in the dermal junction where PPARG is

present in dermal fibroblasts (2011). A logistic regression model with TZD use, BMI, age and HbA1c as independent predictors found that BMI and TZD use were both significant predictors of Vitamin D insufficiency. However one limitation to this study was that other diabetic medications were not controlled for and there was an imbalance of other medications used between the non-TZD and TZD groups (2011).

Another study looked at metformin's possible effect on vitamin D levels (Kos et al, 2012). Since gastrointestinal symptoms creates malabsorption problems of vitamin B12, and vitamin D was hypothesized to be malabsorbed through the same manner, Kos et al (2012) used mixed-effects regression models to investigate vitamin D association with the use of metformin adjusting for age, osteoporosis, and BMI. They found that vitamin D deficiency was not a clinical concern among type 2 diabetes patients taking metformin; no significant difference in 25(OH)D levels was found between the metformin and the non-metformin group (Kos et al, 2012). Finally, a recent study of 499 Saudi patients with type 2 diabetes looked at subsequent lipid profiles by medication status, in conjunction with vitamin D supplementation, and found that those taking rosiglitazone and insulin+oral agents showed a significant improvement in their metabolic profiles after an increase in mean vitamin D levels in all subjects (Alkharfy et al, 2013). Although limited in size and having no physical activity variable accounted for, the study showed that different anti-diabetic therapies can interact and influence circulating levels of vitamin D in diabetes type 2 patients (Alkharfy et al, 2013). A potential effect between diabetes medication and vitamin D status could affect diabetic patients' lipid profiles, among other complications.

Objectives

The objectives of this study are:

- Estimate the disparity of vitamin D deficiency prevalence between Mexican-Americans and non-Hispanic Whites with type 2 diabetes subjects (NHANES 2001-2006).
- Estimate whether any demographic variables, behavioral risk factors, type of diabetes medication used, and cardiovascular-disease related risk factors, which might be associated with vitamin D deficiency (blood pressure, triglycerides, total cholesterol, Hba1c) explain or contribute to any observed vitamin D deficiency disparity between Mexican-Americans and Non-Hispanic Whites with Type 2 Diabetes.

Methods

Study Design

The National Health and Nutrition Examination Survey (NHANES), a stratified, multi-stage, probability sample survey carried out by the National Center for Health Statistics of the CDC, examines a nationally representative sample of US, non-institutionalized civilians each year. Participants were recruited from household clusters, given written informed consent, and interviewed at home with follow-up examinations. NHANES releases this public use data from the personal interviews (demographics, diet, health) and physical examinations (e.g blood, urine samples) from Mobile Examination Centers (MEC) in 2-year cycles. More details about this survey can be found on their website (www.CDC.gov).

Study Population and Variables

For this study, data from 3 NHANES cycles with available serum 25(OH)D data (2001-2002, 2003-2004, 2005-2006) were combined to increase sample size and improve statistical power. Using previous interventional and cross-sectional studies' exclusion criteria based on possible interactions with diabetes risk factors and outcomes of interest, subjects who have taken hydrocortisone/cortisone, steroids, weight-loss drugs, prescription diet pills, taking osteoporosis treatment, anti-epileptic drugs (phenytoin), suffer from liver conditions, and kidney disorders were excluded (Alkharfy et al, 2013). We restricted the analyses to Mexican-American (other Hispanics excluded since previous studies have also focused on Mexican-Americans, the largest and fastest growing Hispanic group in the US) and non-Hispanic White adults' ages 18-85 with diabetes mellitus type 2.

Subjects' dietary supplement and demographic information was obtained from the demographic, dietary, and examination surveys during the home interviews. Blood samples and body measurements (eg. BMI) were taken during the MEC examinations, providing information on serum 25(OH)D, glycohemoglobin, blood pressure, total cholesterol, and total triglycerides. Information on medical history, diabetes diagnosis, medication use, use of weight-loss drugs, and physical activity level during the day were asked during the home interview questionnaire portion. Diabetes mellitus type 2 was defined by age of diagnosis (diagnosed >30 years of age) or currently taking oral-anti diabetic agents/hypoglycemics. These criteria have also been used as the standard for capturing those with diabetes type 2 in past NHANES studies (Wong et al, 2013).

Outcome of Interest

Vitamin D levels were defined as >30 ng/mL (adequate), 16-30 ng/mL (insufficient), and <=15 ng/mL (deficient) (Hollis & Wagner, 2005; Vacek et al, 2011). For logistic regression models, the outcome of interest was specifically the presence of vitamin D deficiency (<=15 ng/mL) versus no deficiency (>15 ng/mL).

Potential Predictors:

Demographics

Age (categorized), gender, and six-month time period of examination were classified as demographic variables and included in all adjusted models (**Model 1-6**). The six-month time period when examination was performed (November 1 through April 30, versus May 1 through October 31) was included as a possible demographic predictor of vitamin D deficiency since summer months provide more sunlight. Dietary supplement use was also adjusted for in all models as a potential confounder since supplement use can affect overall Vitamin D levels and ethnic groups may differ in proportion of their use. Supplement use refers to vitamin, minerals, or other dietary supplements taken in the past month as a proxy for overall supplement use.

Behavioral Risk Factors

BMI (kg/m^2) and average daily physical activity were grouped as behavioral risk factors of vitamin D deficiency. BMI was categorized as follows: normal if BMI <25, overweight if BMI equal to or greater than 25 and less than 30, and obese if BMI >30. Average daily physical activity was assessed using the following variable categories: 1- sit during the day and doesn't walk about very much, 2-stand or walk about a lot during the day, but does not carry or lift things very often, 3-lift light loads or have to climb stairs or hills often, 4- you do heavy work or carry heavy loads.

Cardiovascular-related risk factors

Glycohemoglobin, high triglycerides, high cholesterol, high LDL, hypertension presence, and the presence of a cardiovascular related illness were all grouped as CVD-related risk factors for vitamin D deficiency. Glycohemoglobin (Hba1c), an indicator of diabetes management, with levels below 5.7% categorized as normal or controlled diabetes glucose status was grouped as a CVD-related risk factor since uncontrolled diabetes leads to increased risk of CVD (Heinemann & Freckmann, 2015). Hypertriglyceridemia/high triglycerides was defined as triglyceride levels >200 mg/dL. High cholesterol was defined as total cholesterol levels >240 mg/dL or currently taking prescribed cholesterol medication. High LDL was defined as levels >160 mg/dL. Hypertension was defined as systolic blood pressure >140 mmHG, diastolic > 90 mmHG or currently being treated with hypertension medication. Cardiovascular-related illnesses like congestive heart disease, angina, heart attack, and strokes were grouped together in one indicator variable for presence of cardiovascular-related conditions ('heart') that could be associated with the disparity in vitamin D deficiency.

Thyroid problems and Diabetes Medication

The presence of thyroid problems was also included as a possible confounder of vitamin D deficiency. Thyroid disease and diabetes frequently coexist, with thyroid problems possibly interfering with glycemic control (Witting et al, 2014); In addition, those who suffer from thyroid problems have been linked to vitamin D deficiency (Muscogiuri et al, 2015). Diabetes medication type used will be explored as possible influence on the vitamin D deficiency disparity as well. The diabetes medication type variable was created from NHANES' Questionnaire portion on reported medications. Diabetes medication type was broken down into the following major medication categories (from the drugs classed as anti-diabetic agents): 1-sulfonylureas, 2-

thiazolidinediones, 3- insulin, 4- Metformin, 5- other, 6 – none (no anti-diabetic agent reported). The *other* category is made up of those who reported use of dipeptydyl peptidase 4 inhibitors, alpha-glucosidase inhibitors, amylin analogs, incretin mimetics, and any unspecified anti-diabetic agents.

Methods - Vitamin D Assay Drift Adjustments

Serum 25(OH)D bioavailability makes it a perfect marker for estimating vitamin D levels in the general population, and thus values obtained from NHANES must be accurate for comparability of time trends and assessing prevalence estimates. The National Center for Health Statistics (NCHS) posted an analytical note informing users of NHANES 1988-1994 and 2000-2006 that serum 25(OH)D measures are likely affected by assay fluctuations due to measurement procedure bias and imprecision variations over time (Yetley et al, 2010). Any studies using NHANES 25(OH)D serum measures prior to the adjustment are subject to bias (over or underestimations) and the question of whether the fluctuations are due to actual changes in the population or from measurement variations arises. The NCHS updated and added new assay-adjusted serum 25(OH)D data in November 2010 (Ganji et al, 2011). One study using assay-adjusted data from 2001-2006 NHANES found that waist circumference, systolic blood pressure, and HOMA-IR were inversely related with serum 25(OH)D (Ganji et al, 2011), in adolescents aged 12-19 years. Later measurements for the NHANES 2007 onward serum levels should provide a more precise level given the recommendations by the roundtable experts on improving the quality and consistency across all laboratories measuring 25(OH)D serum (Yetley et al., 2010). In the meantime, several short-term approaches, including readjustments from equations to account for possible assay fluctuations, have been posted by NCHS for interim estimates of

vitamin D estimates needed for public health studies such as this one. This study made use of the readjusted values provided by NCHS.

Statistical Methods

Statistical analyses was carried out by SAS version 9.4 (SAS Institute, Cary, North Carolina) following NHANES guidelines for analyzing data from a stratified cluster design. All continuous variables were tested for normality using SAS's PROC UNIVARIATE procedures, using NHANES weights for each variable. Sample weights, strata, clusters, and variances to account for the sample design were included in the SAS survey procedures for providing descriptive statistics (means, proportions, frequencies). SAS survey procedures were used for carrying out hypothesis testing (Chi-square, T-tests) for differences between categorical and continuous variables. Logistic regressions were performed using the SAS Survey procedures with a dichotomous variable as the outcome of interest (presence or absence of vitamin D deficiency).

For the first objective, the crude vitamin D deficiency disparity between Mexican-Americans and non-Hispanic whites was estimated with t-tests and chi-square tests comparing vitamin D levels (categorized levels of deficiency) between both ethnic groups. An unadjusted logistic regression model was also used to obtain the crude estimate for the disparity.

For the second objective of explaining how much of the crude/racial or ethnic disparity is attenuated by other variables, multivariable logistic regression models were set up for identifying these potential confounders, with race/ethnicity as the main exposure. A forward progression of models was carried out to see how much of the disparity changes with adjustment of potential confounders. Thus, model selection focused on which set of variables explained any disparity,

attenuating or amplifying the main race/ethnicity exposure (eg. being Mexican-American). A difference of 10%, comparing any addition of variable(s) to the crude OR, was used to identify potnential confounders that would help explain the vitamin D deficiency disparity present between Mexican-Americans and non-Hispanic Whites. Additionally, variables with significant associations with vitamin D deficiency were noted in bold (p-value <0.05) in the tables below.

Results

A total of 31,509 respondents were combined from the three NHANES cycles. Only 1,468 of the original total were classified as having type 2 diabetes mellitus. After additional exclusion criteria was applied (age range, no kidney problems, no cortisone use, etc…), only 775 subjects remained that were included in all data analyses, with 292 Mexican-Americans and 483 non-Hispanic Whites. Since variables were categorized, predictors with slightly skewed distributions were not transformed. Vitamin D was categorized for all logistic regression models as the outcome of interest (1 - <=15 ng/mL, 0 – >15 ng/mL). **Table 1** shows the summary characteristics for our study, with significant differences bolded, as indicated by a p-value <0.05.

Overall, Mexican-Americans with diabetes differ significantly from non-Hispanic Whites in various categories, as seen in **Table 1**. Mexican-Americans were slightly younger (mean 55.0 and 62.1 years, respectively), had higher average levels of physical activity (7.5% vs 1.6% at Category 4), higher glycohemoglobin (71.7% vs. 54.7% at elevated level), lower cholesterol (43.6% vs. 53.4% with high cholesterol), less hypertension (51.4% vs 69.1%), less cardiovascular-related illnesses (15.3% and 32.1%), and less thyroid problems (10.0% vs. 15.7%). In addition, less Hispanics had the interview during the sunniest 6-month time period of May-October compared to non-Hispanic Whites (21.0% vs. 64.0%). Dietary supplement use

was also significantly lower in Mexican-Americans compared to non-Hispanic Whites (42.0% vs. 58.6%).

For answering the first objective's goal, **Table 1** shows that vitamin D deficiency is much more prevalent in Mexican-Americans than in non-Hispanic Whites (38.7% vs. 20.0%, p-value <0.0001). In addition, only 5.0% of Mexican-Americans had adequate levels of vitamin D compared with 16.7% for non-Hispanic Whites. The unadjusted logistic regression model estimating the crude vitamin D deficiency disparity in **Table 2a, Model 0** shows an OR of 2.51 for Mexican-Americans vs. non-Hispanic Whites (95% CI 1.66-3.79).

The remaining logistic regression models were run to obtain the differences (eg. possible attenuations) in vitamin D deficiency disparities, adding categories of possible confounders in a forward progression. Results are shown in **Table 2a, 2b,** and **2c.** Next, using the 10% rule, supplement use (shown previously to be a confounder for vitamin D associations) was kept in all remaining models since the OR of our main exposure changed by more than 10% when supplement use was dropped. **Model 1** in **Table 2a** shows that when adjusting for supplement use only, the OR for our main exposure (ethnicity) drops from 2.51 to 2.12 (95%CI 1.37-3.27). That is, supplement use alone appears to account for a 15% drop in risk associated with being Mexican-American. Supplement use remained significant in all other models, as can be seen in **Table2a-2c**. After additionally accounting for demographics in **Model 2**, the OR for our main exposure is further attenuated (OR 1.72, 95% CI 0.97-3.03). That is a 31% decrease in the vitamin D deficiency OR for Mexican-Americans compared to the crude OR. In addition, one can see a significant association between vitamin D deficiency and the male gender variable ($P<0.05$).

In **Model 3**, the OR for the main exposure after adjusting for behavioral risk factors was attenuated to 2.18 (95% CI 1.13-4.23), a 13% attenuation from the crude OR. Those with a BMI greater than or equal to 30 had a significant association with vitamin D deficiency (OR 2.05, 95% CI 1.10-3.82), in addition to those of male gender (OR 0.43, 95% CI 0.26-0.69). **Model 4** shows that after adjusting for possible cardio-vascular related illnesses, the OR for our main exposure also becomes smaller (OR 1.47, 95% CI 0.56-3.87). The vitamin D deficiency disparity is substantially attenuated (41%), in comparison to previous models. In addition, those aged 60-70 also seemed to have a protective effect for vitamin D deficiency (OR 0.31, 95% CI 0.11-0.86).

Model 5 adjusted for possible confounding by the presence of thyroid problems. The main exposure OR was also attenuated to 1.68 (95% CI 0.95-2.97), a 33% change from the crude OR . Male gender (OR 0.34, 95% CI 0.20-0.57) and those aged 71-85 (OR 0.57, 95% CI 0.33-0.99) had significant OR's associated with vitamin D deficiency in this model as well. **Model 6** adjusted for type of diabetes medication used. The OR for our main exposure was greatly attenuated (OR 1.36, 95% CI 0.69-2.70) by 46%, the largest difference from all other models. Those aged 60-70 and those using metformin also seemed to have a slightly significant protective effect on vitamin D deficiency. A full model was also run, adjusting for all potential confounders. The OR for the fully adjusted model was also attenuated by 20% to 2.01 (95%CI 0.48-8.54).

Discussion

Our results suggest that vitamin D deficiency appears to be much more prevalent in Mexican-Americans with type 2 diabetes mellitus, compared to non-Hispanic Whites. This is consistent with previous findings from NHANES showing higher levels of vitamin D for non-Hispanic Whites. However, previous studies have only looked at healthy subjects and not those

with type 2 diabetes mellitus. Vitamin D deficiency in this population may aggravate diabetes complications and may be linked to further cardiovascular related disorders that come about with unmanaged diabetes. There are several possible explanations for this observed disparity. As stated before, Mexican-Americans are more prone to vitamin D deficiency through obesity, as serum 25(OH)D can become sequestered in adipose tissue (Williams et al, 2013). However, in this study, obesity levels were lower in Mexican-Americans compared to non-Hispanic Whites (**Table 1**, 50.8% vs. 61.9% with BMI >30 kg/m^2). Other factors that may reflect this include cultural preferences for certain foods lower in vitamin D levels, biological influences on nutrient bioavailability, and supplement use (Kant et al, 2012). Other explanations for the apparent disparity were explored in the logistic regression models that were conducted, adding groups of possible confounders in a forward progression and seeing if the crude OR for vitamin D deficiency was affected or attenuated by the adjustment of those potential confounders. However, cultural factors, such as food preferences and other lifestyle choices, were not controlled for in this study.

This study also suggests that vitamin D supplementation is a significant confounder in all vitamin D deficiency logistic regression models. After adjusting for supplement use only, the OR for the main exposure of ethnicity was attenuated by ~15% (from 2.51 to 2.12). This is not surprising, given that supplement use, as seen in **Table 1**, was higher in non-Hispanic Whites compared to Mexican-Americans (58.6% vs. 42.0%, p-value=0.0002). Supplement use, according to the NHANES definition, includes any vitamins, minerals, or other dietary supplements taken in in the past month. This could have impacted the overall levels of serum 25(OH)D in subjects, especially if one group reported less use than the other. Nevertheless, supplement use was adjusted in all logistic regression models, along with demographic factors.

This finding may underscore the need for Mexican-Americans to increase use of supplements for maintaining optimum serum 25(OH)D levels.

Models 2, 4, **5, and 6**, which adjusted for demographic factors alone, cardiovascular-related risk factors, thyroid problems, and diabetes medication type (in addition to supplement use) resulted in overall lower ORs for our main exposure (ethnicity). After initially adjusting for demographics and supplement use, both previously found in the literature to be known confounders for vitamin D deficiency, the attenuation was quite significant (31%). Adjusting for behavioral risk factors (**Model 3**) also resulted in a modest attenuation of 13%, compared to the other models' attenuations. This suggests that certain behavioral risk factors, specifically a BMI of >=30 kg/m^2, which had a significant OR, may play a role in the disparity of vitamin D deficiency between Mexican-Americans and non-Hispanic Whites with type 2 diabetes. **Model 5**, which adjusted for confounding by the presence of thyroid problems, previously linked to diabetes mellitus and vitamin D deficiency, also resulted in a large attenuation, 33%. Reasons for this were previously explained, with thyroid problems being a known comorbidity appearing in those with diabetes. This finding is somewhat surprising, given that Mexican Americans (who had lower vitamin D levels in this study) did have a lower prevalence of thyroid problems compared to non-Hispanic whites (10.0% vs. 15.7%). However, information on the severity and type of thyroid disorders (eg. hypothyroidism, hyperthyroidism, etc…) was not taken into account in this analysis.

The two models (**Model 4** & **6)** that adjusted for cardiovascular-related risk factors and diabetes medication type resulted in the largest attenuations of the crude OR (41% and 46%, respectively). Cardiovascular-related risk factors have been linked to the presence of vitamin D deficiency, specifically from factors such as dyslipidemia (high triglycerides, high LDL, etc..). In

this study, Mexican-Americans, compared to non-Hispanic Whites, did have a higher prevalence for hypertriglyceridemia (44.6% vs. 32.2%) and high LDL (12.5% vs. 8.9%). Although these differences, shown in **Table 1**, were not statistically significant, they still reveal a possible explanation for the large attenuation in the OR adjusting for cardiovascular-related factors. Lack of significance could just be a result of lower number of observations in strata. This large attenuation from the adjustment of cardiovascular-related risk factors underscores the previously found associations with vitamin D deficiency. However, the CVD risk factor role in explaining the deficiency disparity had not been previously explored. In addition, the fact that CVD risk factors, such as dyslipidemia, are highly prevalent in (and directly associated with diabetes) Mexican-Americans underscores how disparities in one health condition may reveal more disparities, in this case, a greater vitamin D deficiency compared to non-Hispanic Whites (Rodriguez et al, 2014).

Adjustment of diabetes medication type resulted in the greatest attenuation of the crude OR, a reduction of 46%. Type of diabetes medication, as previously explained, may then play a role in vitamin D and the observed disparity in this study. Differences in medication type used between Mexican-Americans and non-Hispanic whites were not significant, however. Thiazolidinedione use was slightly higher in non-Hispanic whites compared to Mexican-Americans, although not significantly (5.2% vs. 8.2%). Thiazolidinedione users have been found to be linked with higher levels of serum 25(OH)D through a known biological mechanism of the PPAR receptor (Chakreeyarat et al, 2011), as previously mentioned. Larger number of observations or an overall increase in sample size could have revealed potential significant differences between these groups, however. This, along with other significant differences in type of medication used, could further reveal how the type of medication used between Mexican-

Americans and non-Hispanic whites can play a role in the observed vitamin D deficiency disparity.

The fully adjusted model (**Model 7**) also showed an attenuation of 20% (from 2.51 to 2.01). However, categorical variables, such as average level of daily physical activity, had to be regrouped in order for convergence of the model to be reached. In addition, larger confidence intervals in variable ORs reveals that the model may need more observations for better conclusions to be made.

Conclusion

In conclusion, this study suggests that those with type 2 diabetes mellitus have overall low levels of vitamin D. Adequacy was low for both Mexican-Americans and non-Hispanic Whites, though lower in the former group. A disparity is seen through significant differences in vitamin D deficiency between both ethnic groups. This disparity is also continuously present through the higher ORs (>1.00) linking Mexican-Americans with type 2 diabetes melltius to vitamin D deficiency after running all logistic models, unadjusted and adjusted. This confirms previous studies' findings on the disparity of vitamin D deficiency between non-Hispanic Whites and Hispanics (Gupta et al, 2012; Martins et al, 2007). Potential factors (eg. BMI, cardiovascular-related risk factors, etc…) that could help explain the disparity did result in various attenuations of the crude OR. In this study, adjusting for demographic factors and supplement use in all models, in addition to behavioral risk factors, cardiovascular-related risk factors, thyroid comorbidity, and diabetes medication type all attenuated the disparity. Supplement use in all logistic regression models was a significant confounder and greatly attenuated the crude OR, suggesting that it plays a huge role in the vitamin D deficiency disparity. In addition, cardiovascular-related risk factors and type of diabetes medication used

also greatly attenuated the OR, highlighting what has been found in previous studies but now in terms of explaining a possible disparity of vitamin D deficiency.

Strengths of this study include the overall estimation of the vitamin D deficiency disparity between Mexican-Americans and non-Hispanic Whites with type 2 diabetes mellitus, a population that hasn't been specifically studied before using NHANES data. Since vitamin D deficiency has been associated with various pre-disease conditions (Gupta et al, 2012), those with diagnosed diabetes may suffer more from the effects of vitamin D deficiency. We also investigated possible explanations for observed disparities between Mexican-Americans and non-Hispanic Whites with type 2 diabetes mellitus, finding that supplement use was consistently associated with vitamin D deficiency, and that CVD-related risk factors and diabetes medication greatly attenuated the vitamin D deficiency disparity. In addition, this study made use of assay-adjusted serum 25(OH)D measurements posted by NCHS in 2011. Previous NHANES studies had not used assay-adjusted measurements and thus, could have over or under-estimated their findings. The use of NHANES data also assures that it is a nationally representative sample and provides detailed information on the variables used for analyses.

There are a number of limitations in this study that could affect the interpretation of the results. NHANES is a cross-sectional study that collects vast information on several topics over two-year cycles. The study design, thus, does not allow for temporality or causality to be established, only associations. This warrants further investigations by randomized controlled trials and other studies that can more clearly investigate temporal associations. Another limitation was that several variables were categorized together, which could have hidden some associations. Cardiovascular-related illnesses (angina, strokes, heart-attacks,etc…) were grouped together in one indicator variable, for example. In addition, several other confounders that could

have provided more information on our questions were not adjusted for. More variables on behavioral/lifestyle risk factors were not included, such as occupation type (which may increase exposure to the sun), due to differences in the way the responses were coded throughout the cycles. Due to a large number of variables entered in models, a larger sample size could have also revealed more significant associations and larger attenuations of our crude OR. Future studies should consider more lifestyle risk factors that could help explain the vitamin D deficiency disparity. Geographical latitude, which might have contributed to some of the vitamin D deficiency found in NHANES data, was also not accounted for. NHANES geocoding information is limited access only and was not obtained for this analysis.

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TABLES

Table 1. Characteristics of Participants with Type 2 Diabetes, NHANES 2001-2006			
n (%) or mean (SD)			
	Mexican-American	Non-Hispanic White	P-value
N	292	483	
Vitamin D, ng/mL	18.0(0.66)	22.6(0.42)	**<0.0001**
Deficient (<=15 ng/mL)	102 (38.7%)	76 (20.0%)	**<0.0001**
Insufficient (16-30 ng/mL)	155 (56.3 %)	271 (63.3%)	
Adequate (>30 ng/mL)	13 (5.0%)	67 (16.7%)	
Age, years	55.0 (1.37)	62.1 (0.88)	**<0.0001**
18-39	11 (9.9%)	13 (3.5%)	**<0.0001**
40-59	80 (52.0%)	110 (38.3%)	
60-70	133 (25.8%)	120 (26.3%)	
71-85	68 (12.4%)	240 (31.5%)	
Gender (Male)	147 (46.8%)	265 (51.3%)	0.1937
Average level of PA each day			**0.0015**
1 – sits during day /doesn't walk very much	76 (23.3%)	196 (35.7%)	
2 – stand/walk about a lot during the day	169 (54.3%)	228 (50.2%)	
3 –lift load or have to climb stairs/hills often	32 (14.8%)	53 (12.5%)	
4 – do heavy work /carry heavy loads	15 (7.5%)	6 (1.6%)	
Dietary supplement use	124 (42.0%)	285 (58.6%)	**0.0002**
0-<25 (normal)	46 (16.1%)	59 (10.4%)	0.0733
25-<30 (overweight)	103 (33.1%)	130 (27.8%)	
>=30 (obese)	120 (50.8%)	224 (61.9%)	
Glycohemoglobin, %	8.0 (0.17)	6.9 (0.08)	**<0.0001**
<5.7%	21 (8.3%)	51 (13.1%)	**0.0012**
5.7-6.4%	54 (20.1%)	140 (32.2%)	
>=6.5%	199 (71.7%)	229 (54.7%)	
Hypertriglyceridemia (>200 mg/dL)	48 (44.6%)	53 (32.3%)	0.1102
High Cholesterol (>=240 mg/dL or taking meds)	123 (43.6%)	222 (53.4%)	**0.0454**
High LDL, > 160 mg/dL	11 (12.5%)	11 (8.9%)	0.4851
Hypertension	164 (51.4%)	293 (69.1%)	**0.0003**
Cardiovascular-related illness**	51 (15.3%)	192 (32.1%)	**<0.0001**
Thyroid problems	27 (10.0%)	78 (15.7%)	**0.0410**
Diabetes Medication Type***			
Sulfonylureas	58 (21.8%)	80 (17.7%)	0.2026
Thiazolidinediones	9 (5.2%)	26 (8.2%)	
Insulin	11 (7.6%)	13 (2.4%)	
Metformin	46 (23.0%)	69 (25.5%)	
Other****	13 (6.3%)	32 (11.1%)	
None (no anti-diabetic agent reported)	57 (36.1%)	119 (35.0%)	
Season			**<0.0001**
Nov-April 30	236 (79.0%)	157 (35.9%)	
May-Oct 31	45 (21.0%)	279 (64.0%)	

Table 2a. Multiple Logistic Regressions for Vitamin D Deficiency Risk Factors between Mexican Americans and Non-Hispanic Whites			
Vitamin D Deficiency (<=15 ng/mL)			
	Model 0 (Unadjusted)	Model 1	Model 2
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ethnicity (Main Exposure):			
Mexican-Americans	**2.51 (1.66-3.79)**	**2.12 (1.37-3.27)**	1.72 (0.97-3.03)
NH Whites	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Demographics			
Gender (Male)	-	**-**	**0.36 (0.22-0.59)**
Age, years			
18-39	-		0.35 (0.09-1.36)
40-59	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
60-70	-	-	0.61 (0.33-1.10)
71-85	-	**-**	0.58 (0.33-1.01)
Season	-	-	0.75 (0.41-1.39)
Supplement Use (confounder?)*		**0.28 (0.19-0.42)**	**0.22 (0.14-0.35)**
* Model with either both or 1 confounder only showed a difference >10% when comparing models with supplement use and without (unadjusted). Controlling for supplement use, OR is 2.1. Without controlling, OR is 2.52 (unadjusted).			

Table 2b. Multiple Logistic Regressions for Vitamin D Deficiency Risk Factors between Mexican Americans and Non-Hispanic Whites			
Vitamin D Deficiency (<=15 ng/mL)			
	Model 3	Model 4	Model 5
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Ethnicity **(Main Exposure):**			
Mexican-Americans	**2.18 (1.13-4.23)**	1.47 (0.56-3.87)	1.68 (0.95-2.97)
NH Whites	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Demographics			
Gender (Male)	**0.43 (0.26-0.69)**	0.40 (0.15-1.10)	**0.34 (0.20-0.57)**
Age, years			
18-39	0.36 (0.08-1.54)	0.29 (0.03-2.70)	0.34 (0.09-1.33)
40-59	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
60-70	0.61 (0.33-1.12)	**0.31 (0.11-0.86)**	0.60 (0.33-1.07)
71-85	0.73 (0.41-1.32)	0.50 (0.16-1.57)	**0.57 (0.33-0.99)**
Season	0.79 (0.43-1.48)	0.50 (0.17-1.50)	0.75 (0.41-1.39)
Supplement use	**0.21 (0.14-0.33)**	**0.20 (0.07-0.59)**	**0.22 (0.14-0.34)**
Behavioral Risk Factors			
BMI group			
<25	0.87 (0.45-1.68)	-	-
25-<30	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
>=30	**2.05 (1.10-3.82)**	-	**-**
Physical Activity			
0	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
1	0.62 (0.34-1.14)	-	-
2	0.53 (0.24-1.16)	-	-
3	0.32 (0.03-3.94)	-	-
Cardiovascular-related Risk Factors			
Elevated Glycohemoglobin, >5.7 %	-	0.56 (0.17-1.85)	-
High Triglycerides, >200 mg/dL	-	2.11 (0.87-5.14)	-
High Cholesterol, >240 mg/dL	-	1.17 (0.46-2.99)	-
High LDL, >160 mg/dL	-	1.49 (0.31-7.13)	-
Hypertension	-	1.09 (0.43-2.82)	-
Cardiovascular-related illness	-	2.54 (0.84-7.73)	-
Comorbidity			
Thyroid	-	-	0.69 (0.30-1.63)
Diabetes Medication Type			
Sulfonylureas	-	-	-
Thiazolidinediones	**-**	**-**	-
Insulin	-	-	-
Metformin	-	**-**	-
Other	-	-	-
None	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)

Table 2c. Multiple Logistic Regressions for Vitamin D Deficiency Risk Factors between Mexican Americans and Non-Hispanic Whites		
Vitamin D Deficiency (<=15 ng/mL)		
	Model 6	Model 7
	OR (95% CI)	OR (95% CI)
Ethnicity **(Main Exposure):**		
Mexican-Americans	1.36 (0.69-2.70)	2.01 (0.48-8.54)
NH Whites	1.00 (Ref)	1.00 (Ref)
Gender (Male)	**0.38 (0.22-0.68)**	0.43 (0.11-1.65)
Age, years		
18-39	0.39 (0.10-1.54)	0.51 (0.02-14.06)
40-59	1.00 (Ref)	1.00 (Ref)
60-70	**0.53 (0.32-0.86)**	0.24(0.04-1.68)
71-85	0.57 (0.31-1.05)	0.37 (0.08-1.70)
Season	0.73 (0.40-1.34)	0.65 (0.17-2.57)
Supplement use	**0.19 (0.10-0.35)**	**0.21 (0.04-1.00)***
Behavioral Risk Factors		
BMI group		
<25		0.45 (0.12-1.71)
25-<30		0.57 (0.14-2.24)
>=30		0.98 (0.08-11.88)
Physical Activity**		
0		1.00 (Ref)
1		0.57 (0.14-2.24)
2		0.98 (0.08-11.88)
Cardiovascular-related Risk Factors		
Elevated Glycohemoglobin, >5.7 %		
High Triglycerides, >200 mg/dL		1.60 (0.58-4.43)
High Cholesterol, >240 mg/dL		3.38 (0.82-13.97)
High LDL, >160 mg/dL		2.06 (0.22-18.94)
Hypertension		0.53 (0.17-1.69)
Cardiovascular-related illness		2.45 (0.53-11.29)
Comorbidity		
Thyroid		0.29 (0.05-1.51)
Diabetes Medication Type		
Sulfonylureas	0.76 (0.33-1.74)	0.51 (0.13-1.92)
Thiazolidinediones	1.28 (0.38-4.30)	0.09 (0.01-1.16)
Insulin	2.12 (0.66-6.75)	2.01 (0.12-33.89)
Metformin	**0.44 (0.20-0.97)**	0.27 (0.06-1.12)
Other****	0.76 (0.25-2.31)	0.90 (0.18-4.53)
None	1.00 (Ref)	1.00 (Ref)

*P-value= 0.05

**Physical Activity Categories 3 & 4 were combined due to low # of observations in full model

SUMMARY & FUTURE HEALTH IMPLICATIONS

Findings from this study reveal that there is a vitamin D deficiency disparity present between Mexican-Americans and non-Hispanic whites with type 2 diabetes mellitus. Since vitamin D deficiency has been linked to various skeletal and non-skeletal (cardio-metabolic) risk factors, this disparity in the deficiency should be a target for healthcare providers when addressing diabetes management. The analysis also points to various confounders that may explain the observed disparity. There was a significant association for all adjusted models between supplement use and vitamin D deficiency, along with a great attenuation of the crude OR. This also highlights the facts that Mexican-Americans had a lower prevalence of supplement use overall, a finding that healthcare providers should consider. A great attenuation of the crude OR by adjustment of cardiovascular-related risk factors and type of diabetes medication suggests that these sets of confounders should also be further explored in future clinical studies.

Since the sample size of this study did not have as many observations for various stratum, including type of medication used, and since cardio-vascular related risk factors were grouped together in one indicator variable, future clinical studies should also consider this and try to address this when trying to further elucidate the actual causes of the observed vitamin D deficiency disparity in this observational study.