I, Chad Bitler, hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled: 
Vitamin D and Markers of Glucose Metabolism

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Vitamin D and Markers of Glucose Metabolism

A thesis submitted to the
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in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Nutritional Sciences
College of Allied Health Sciences

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ABSTRACT

Objective: To examine whether serum vitamin D levels are related to markers of glucose metabolism as measured by A1C, fasting glucose, and fasting insulin,


Subjects: Non-diabetic adults, ages 20 years and older, with measures of serum vitamin D, fasting glucose, fasting insulin, and A1C were included in the study. Race/ethnicity, as reported by the subject, was categorized as Mexican American (N = 254), other Hispanic (N = 46), non-Hispanic White (N = 579), African American (N = 278), & multi-racial (N = 57)

Methods: Data collected from 1214 adult participants in the National Health and Nutrition Examination Survey (NHANES) 2005 – 2006 was accessed via the public domain and analyzed using SAS Enterprise software, version 6.1. Pearson correlation, odds ratio, and chi-squared tests were performed to determine the strength and significance of the relationship between levels of serum vitamin D and A1C, fasting glucose, and fasting insulin status.

Results: Serum vitamin D was inversely correlated with A1C (r = -0.12; p = < 0.0001), fasting glucose (r = -0.08; p = 0.005), and fasting insulin (r = -0.18; p = < 0.0001), across
the study population. For the total study population, the likelihood of having an abnormal marker of glucose metabolism with adequate serum vitamin D (≥ 30 ng/mL) was reduced for fasting insulin, (OR = 0.40; 95% CI = 0.29 – 0.54) and A1C (OR = 0.67; 95% CI = 0.44 – 0.99) but not significant for glucose (OR = 0.78; 95% CI = 0.57 – 1.06). When racial and ethnic groups were subdivided, the only marker that remained significant was fasting insulin in the Caucasian (OR = 0.50; 95% CI = 0.34 – 0.73) and Mexican-American (OR = 0.16; 95% CI = 0.07 – 0.39) groups.

**Conclusion:** The results of this study show an inverse association between serum vitamin D and A1C, fasting glucose, and fasting insulin. Adequate levels of serum vitamin D (≥ 30 ng/mL) was also found to be associated with reduced risk of impaired fasting insulin and A1C in the total population. In subgroups, the odds of having an abnormal insulin level was only significant in Mexican-American and non-Hispanic white participants. This data extends previous research and provides new evidence that serum vitamin D may play a role in glucose metabolism, which may have implications for the pathogenesis of type 2 diabetes.
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Introduction

The incidence of type 2 diabetes mellitus (T2DM) is increasing at an alarming rate both in the United States and worldwide. According to the American Diabetic Association (ADA), in 2012, diabetes affected 29.1 million people, or 9.3% of the US population, up from 25.8 million (8.3%) in 2010. In 2012, 86 million, or 37% of adults, age 20 and over, had prediabetes, up from 79 million in 2010. The most recent World Health Organization (WHO) estimate from 2010 showed that 347 million people worldwide currently have diabetes.

Diabetes is the leading cause of kidney failure, non-traumatic lower limb amputations, and new cases of blindness in the United States, as well as a major cause of heart disease and stroke. Diabetes is also the 7th leading cause of death in the US, based on the death certificates filed in 2010 with diabetes listed as the underlying cause of death. Diabetes prevalence varies by ethnic group. Of adults, age 20 and up, diabetes prevalence occurs most often in non-Hispanic blacks (13.2% of the population), followed by Hispanics (12.8%), and Caucasians (7.6%).

Several lifestyle factors have been associated with the incidence of diabetes. The increase in the prevalence of diabetes has occurred concurrently with the prevalence of obesity, which is known to be a strong risk factor for the development of diabetes. Therefore it is critical to identify factors that are independent of weight that will contribute to abnormal glucose metabolism and insulin resistance, beyond the difficult process of weight loss. With insulin resistance being a known risk factor for T2DM, understanding the role of nutritional, as well as other modifiable risk factors that may contribute to insulin resistance may become increasingly important. It has been proposed
that vitamin D may play an important role in the development of insulin resistance and diabetes. The goal of this research is to determine if there is any relationship between vitamin D status and markers of glucose metabolism, which may play a role in the development of T2DM.

Review of the Literature

Pathogenesis of Type 2 Diabetes Mellitus

Some of the most well recognized independent risk factors for T2DM are age, obesity, and family history. In addition, there have been genes identified that have been found to be associated with T2DM, including those that code for β-cell function (ability to sense blood glucose levels, insulin synthesis, and insulin secretion), insulin receptors, hepatic synthesis of glucose, glucagon synthesis, and cellular responsiveness to insulin stimulation. Abnormal functioning of these genes, combined with lifestyle factors and influences result in the basic pathophysiologic mechanisms of T2DM: insulin resistance and impaired function of pancreatic β-cells. Although many individuals with risk factors for T2DM (including obesity and hypertension) are insulin resistant, only those individuals who develop β-cell dysfunction (and therefore a relative deficiency in insulin) will develop T2DM.

Insulin resistance is defined as a suboptimal response of insulin-sensitive tissues (especially muscle, and adipose tissue) to insulin. Several mechanisms are involved in abnormalities of the insulin-signaling pathway and can contribute to insulin resistance. These can include an abnormality of the insulin molecule, high amounts of insulin
antagonists, down-regulation of insulin receptors, and alteration of glucose transporter proteins (GLUT).

Insulin resistance may be compensated for by an increase in insulin output by the β-cells. This hyperinsulinemia may prevent the clinical onset of T2DM for many years. The emergence of T2DM occurs as the β-cells start to become damaged or stop functioning properly. The development of β-cell dysfunction then leads to a relative deficiency of insulin activity. The dysfunction may be caused by a decrease in β-cell mass, abnormal function of the β-cells, or some combination of both. In T2DM, there is a progressive decrease in both the weight and number of β-cells, and several different mechanisms have been implicated. β-cells are extremely sensitive to high levels of glucose and free fatty-acids, and under these glucolipotoxic conditions, β-cells undergo apoptotic cell death. A variety of pro-inflammatory cytokines, such as TNF-α, IL-12, and IL-2, have also been shown to be toxic to β-cells. High levels of these circulating cytokines promote programmed cell death in the β-cells.

**Prediabetes**

Prediabetes (also referred to as impaired glucose tolerance or impaired fasting glucose) is when blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes. A person who has been diagnosed with prediabetes is at increased risk for developing T2DM as well as for other cardiovascular related diseases, such as heart disease or stroke. Prediabetes often goes undiagnosed due to the lack of symptoms. Without lifestyle changes or medications, people with prediabetes are at an increased risk for development of T2DM, however, with modest weight loss and
moderate physical activity, diabetes can be delayed or possibly prevented. Persons with prediabetes should be checked for the development of T2DM every one to two years. Since being able to modify lifestyle could have a potential positive effect on outcomes associated with prediabetes, this stage could be of great importance in reducing T2DM prevalence.

**Diagnosis of Diabetes and Prediabetes**

Biochemical measures are used for the diagnosis of prediabetes and diabetes.

*\( A1C \)*

Glycated hemoglobin (A1C) is when hemoglobin, a protein within red blood cells that carries oxygen throughout your body, joins with glucose in the blood, becoming “glycated.” The amount of glucose that combines with hemoglobin is directly proportional to the total amount of glucose in the bloodstream. Because red blood cells survive for 8 – 12 weeks, measuring A1C can be used to reflect average blood glucose levels over that duration. Thus, A1C is indicative of long-term blood glucose levels. The normal levels for A1C are between 4% and 5.9%. Values between 5.9% and 6.4% indicate an increased risk of diabetes (prediabetes) and values 6.5% and above could indicate the development of diabetes.

*Fasting Glucose*

Fasting glucose is measured when a person has fasted for a minimum of 8 hours and is used to screen for abnormalities in glucose metabolism. The normal range for blood glucose is less than 100 mg/dL (100 milligrams of glucose per deciliter of blood).
A fasting glucose in the range of 100 mg/dL to 125 mg/dL is indicative of impaired fasting glucose or prediabetes, and above 126 mg/dL is the diagnostic cut-point for diabetes.  

Fasting Insulin

Fasting insulin is measured to determine the amount of insulin in the blood after 8 hours of fasting. The gold standard method for determining insulin resistance is the “hyperinsulinenemic euglycemic clamp,”13 which measures the amount of glucose needed to compensate for an increased insulin level without causing hypoglycemia. In this test, insulin is administered through a peripheral vein. To counterbalance the infusion of insulin, a 20% glucose solution is also administered. Blood glucose levels are then checked at regular intervals. The rate of glucose infusion during the last thirty minutes of the test determines the insulin sensitivity. If high levels are needed (7.5 mg/min or above) then the individual is insulin sensitive. Low levels (below 4.0 mg/min) suggest insulin resistance and levels between 4.0 and 7.5 are non-suggestive but may be used as an early sign of insulin resistance.9

Not all of these blood tests are required for diagnosing diabetes and others, such as the oral glucose tolerance test or the random plasma glucose test may also be utilized for diagnosis.

Vitamin D

The discovery of vitamin D in the early twentieth century and its role in bone health led to a stark decline in the incidence and prevalence of rickets, a disease which,
plagued children in industrializing nations around the globe. In subsequent decades, the greater understanding of vitamin D biology coupled with the rapid increase in epidemiological and clinical studies, which examined the relationships between vitamin D status and various diseases, has led to a greater interest in the complex roles in which vitamin D may play in health outcomes. A distinguishing aspect of vitamin D is that it can be synthesized by humans through the action of sunlight on exposed skin. Thus an understanding of vitamin D as both a hormone and a nutrient are essential in determining its role in health outcomes. Vitamin D and calciferol refer collectively to vitamin D$_2$ (ergocalciferol) and vitamin D$_3$ (cholecalciferol). Vitamin D$_3$ is the form that is produced in the skin of vertebrate animals when exposed to ultraviolet (UV) irradiation, whereas vitamin D$_2$ is produced from the precursor ergosterol by some phytoplankton, yeast, invertebrates, and fungi in response to UV irradiation. Differences in the side chains is what characterizes the two molecules, however the biological activity of vitamins D$_2$ and D$_3$ are considered to be equivalent in regards to correction of vitamin D deficiency.

**Endogenous Synthesis and Metabolism of Vitamin D**

Vitamin D can be synthesized by humans through a delicately regulated multistep process that begins in the innermost layers of the skin, the stratum basal and the stratum spinosum. In the skin, 7-dehydrocholesterol (7-DHC) is converted to previtamin D$_3$ when exposed to ultraviolet B (UVB) photons from the sun. Previtamin D$_3$ then undergoes a thermal isomerization of its double bonds to form vitamin D$_3$.

The synthesis of vitamin D$_3$ is impacted by the intensity of irradiation reaching the dermis, and the length of exposure. The time of day, season, latitude, and amount of
pollution all have dramatic effects on the amount of UVB rays that reaches the earth’s surface. ¹¹

Skin pigmentation is another key area that may affect the synthesis of vitamin D through exposure to UVB irradiation. Skin color is mainly due to pigments called melanins, which are produced by melanocytes in the epidermis. Melanins can act as photoprotectants by absorbing UVB radiation. An individual with dark skin pigmentation may require about ten times longer exposure to sunlight in order to produce the same amount of vitamin D₃ in his or her skin as a light-skinned person. ¹²

Other factors that influence endogenous synthesis of vitamin D include: use of sunscreen, extensive body covering, and declines in organ and tissue function due to aging. Less studied factors effecting synthesis include, effects of altitude, regular outdoor activities, and poorly understood genetic factors that may influence the many steps in vitamin D metabolism and action. ¹¹

Uptake of Dietary Vitamin D

In addition to endogenous synthesis, vitamin D can also be attained from the diet as vitamin D₂ and D₃. Since vitamin D is fat-soluble, the vitamin is absorbed with other lipids in the intestine, a process facilitated by pancreatic lipase and bile. Upon absorption, vitamin D appears in the chylomicrons that are released by the enterocytes and enters the lymphatic system, which drains into the venous bloodstream. Lipoprotein lipase, particularly that which is found in adipose tissue, acts upon chylomicron lipids and may result in a fraction of the vitamin D being taken up by fat cells. This observation suggests a mechanism by which increased adiposity causes sequestering of
vitamin D and is related to lower vitamin D status. Sequestration by adipose tissue leads to stores, which cannot be actively used in times in need. Therefore, obese individuals may require increased intakes of vitamin D in order to achieve serum concentrations of 25-hydroxyvitamin D (25(OH)D) comparable to those found in lean individuals. Following depletion of triacylglycerols, the cholesterol-rich chylomicron remnants which still contain a significant fraction of the absorbed vitamin D are subsequently taken up by the liver, where it is hydroxylated to form 25-hydroxycholecalciferol (25(OH)D). The plasma 25(OH)D level is currently considered to be the best measure of vitamin D status since it has a relatively long half life of 15 days, and reflects vitamin D that is acquired both cutaneously and from dietary intake. For the purpose of this study, vitamin D and 25(OH)D will be used interchangeably. The subsequent metabolism of 25(OH)D to 1,25-dihydroxy-vitamin D (1,25(OH)\textsubscript{2}D) occurs in the kidneys and is tightly regulated. The biologically active form of vitamin D, 1,25(OH)\textsubscript{2}D, is responsible for carrying out most, if not all, biological functions of vitamin D. 1,25(OH)\textsubscript{2}D, however, is a poor indicator of status due to its short half life of approximately 15 hours.

**Genomic Actions of Vitamin D**

The discovery of the vitamin D receptor (VDR) as a member of the steroid hormone superfamily showed that the VDR has many characteristics similar to receptors for thyroid hormone, estrogen, testosterone, and retinoids. The VDR is found primarily in the nuclei of target cells, and has a high affinity for 1,25(OH)\textsubscript{2}D (calcitriol) hormone. Once the calcitriol is bound to the VDR, the VDR acts as a transcription factor by
phosphorylating the calcitriol so it can form heterodimers, which can then interact with coregulatory proteins, allowing the complex to bind to specific genomic sequences in the promoter regions of genes. The VDR-specific binding sites are called vitamin D-responsive elements (VDREs).

In recent decades, it has been found that the VDR is present in many tissues, such as bone, skin, intestine, kidneys, brain, eyes, pancreatic islets (β-cells), immune cells, muscle, and adipose tissue, to name a few.\(^\text{14}\) Knowing that the VDR acts as a transcription factor, in addition to the presence of VDREs in the promoter regions of over 3% of genes in the human genome, has increased research efforts to better understand the roles of vitamin D in the tissue and organs where the VDR is expressed, including the pancreatic β-cells. VDREs are found in genes involved in a diverse range of processes, including the regulation of cell proliferation, differentiation, and apoptosis.\(^\text{15}\)

**Defining Vitamin D Deficiency**

There is lack of agreement regarding the serum concentrations of 25(OH)D associated with optimal overall health.\(^\text{15}\) This is primarily due to the lack of randomized controlled trials for the non-musculoskeletal effects of vitamin D. Therefore, most public health recommendations are based primarily on serum levels needed to provide adequate bone health, i.e. limiting fractures, osteoporosis, and rickets.\(^\text{16}\) There have been studies showing evidence of health benefits in the 20 – 50 ng/mL range and therefore, some organizations base their recommendations on those results; however, the results have been inconsistent.\(^\text{15,17}\) A leading researcher in the field, Dr. Robert Heaney from Creighton University stated the likely reasons for the inconsistencies include the
“intrinsic smallness of nutrient effects, as well as failure of trial designers to give adequate attention to starting vitamin D status and to adequacy of dose.”¹⁸ He also states, “systematic reviews have failed to use dose or starting level as a criteria for study inclusion.” The results of these issues are null studies on the one hand, and, on the other, meta-analytic aggregate effects become minimized.¹⁵

Organizations in the United States have different recommendations for serum vitamin D levels (Table 1). The definition of the lower limit for the optimal range of serum vitamin D is extremely controversial. Reference ranges vary considerably throughout the world. For example, in the United Kingdom, where there is relatively less light exposure and less fortified food than in the US, the lower limit to be considered non-deficient is 3 ng/mL. In contrast, in the US that number is widely considered to be above 12 ng/mL. The lack of standardization has made it difficult to define the levels of vitamin D that could be considered deficient. This has led to recommendations to forgo normal ranges for serum vitamin D all together and instead use a “target” concentration of vitamin D, which would be derived from parathyroid hormone (PTH) measurements. One of the most well known, and understood functions of vitamin D, is its role in calcium absorption. With vitamin D deficiencies, adaptations take place in order to maintain calcium homeostasis. The first adaptation is an increase of PTH levels, which increases serum calcium by resorption from the calcium in bones. This makes PTH an early indicator of vitamin D deficiency.¹⁸ Using PTH as a marker for vitamin D deficiency eliminates geographical and seasonal variations that affect population-based normal ranges. Using PTH as a marker for vitamin D deficiency does have its drawbacks, which is why it’s not the universal standard currently used for determining optimal vitamin D
levels. First, PTH is degraded quickly by peptidases in the blood, giving it a short half-life of 3-5 minutes. As of now, there are no assays that are specific for biologically active PTH, which makes collection complex and requires proper precaution, handling, and technique. Lastly, the set point of PTH levels in response to low levels of serum calcium varies among individuals and is the result of a complex interaction of factors such as age, gender, genetics, renal function, calcium intake, and phosphate and magnesium status.\(^{19}\) Studies have shown that PTH levels begin to plateau in adults who have serum 25(OH)D levels between 30-40 ng/mL and these findings are consistent with the threshold for hip and non-vertebral fracture prevention from a recent meta-analysis of double-blind randomized controlled trials with oral vitamin D.\(^{16}\) Studies like these have led to recommendations for the optimal lower limit of vitamin D to be in the 30-32ng/mL range, and thus is the recommended levels of the Endocrine Society. Since the study population used for the purpose of this thesis is multi-ethnic and representative of various geographical regions from around the US, I have elected to use 30ng/mL as the cut point for levels of vitamin D required to be considered sufficient, in order to account for those variations.

<table>
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<th>Table 1. US Organizations That Have Vitamin D Recommendations</th>
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Factors Associated With Vitamin D and Glucose Metabolism

Ethnicity

The prevalence of diabetes varies by ethnic group as does the levels of serum 25(OH)D. According to the ADA, African Americans have a higher prevalence of T2DM compared to non-Hispanic whites at a rate of 13.2% versus 7.6% respectively. African Americans also have the highest rates of vitamin D deficiency, based on their serum levels, compared to that of any other Americans. Darker skin pigmentation, which makes it more difficult to absorb UV rays in order to synthesize vitamin D subcutaneously, is a likely factor, but other contributing factors such as obesity rates (African Americans have 51% higher and Hispanics have 21% higher rates of obesity when compared to Caucasians), which could lead to sequestration of vitamin D in adipose tissue, should also be accounted for.

Body Mass Index (BMI)

In order to take into account the obesity factor across all races, variables BMI and android fat mass will be adjusted for. BMI is an indicator of adiposity, calculated as weight in kilograms divided by the square of height in meters, was categorized as “underweight” (<18.5 kg/m²), “normal weight” (18.5 – 24.9 kg/m²), “overweight” (25 – 29.9 kg/m²), and “obese” (≥30 kg/m²) using WHO criteria. BMI, however, has some limitations, as it does not distinguish between weight due to excess fat, bone mass, or muscle. For instance, muscular individuals, such as highly trained athletes may have a high BMI due to increased muscle mass.
Android Fat Mass

To account for the limitations with BMI, android fat mass via dual-energy x-ray absorptiometry (DXA) is utilized as a more consistent measurement of body fat. DXA is one of the most widely accepted methods of measuring body composition, due in part to its speed, ease of use, and low radiation exposure. In 2005-2006, whole body DXA scans were administered in the NHANES mobile examination center. The android area is roughly the area around the waist between the mid-point of the lumbar spine and the top of the pelvis. Fat deposition in the android region is associated with increased risk of cardiovascular disease, hypertension, insulin resistance, and T2DM. Abdominal fat percentage is dependent on sex and age, however a study by Mousa et al. investigated the percentage of abdominal fat associated with predicting metabolic syndrome and found that for men an abdominal fat percentage of 35.4% and for women 45.3% had both a high specificity and sensitivity for predicting metabolic syndrome.

Gender

According to the Center for Disease Control men have a higher prevalence of diabetes overall when compared to women.

Age

Age is a non-modifiable risk factor for the development of T2DM. In 2011, 63% of adults (ages 18 – 79 years) diagnosed with diabetes were between the ages of 40 and 64 years. Possible reasons for the increase in diabetes in this age group could include loss of muscle mass, increase in fat mass, and reduction in exercise as we age.
As we age it also becomes more difficult for us to efficiently synthesize vitamin D through our skin, leading to an increase in risk for vitamin D deficiency, especially in the elderly.  

*Physical Activity Level*

Increased physical activity can lower diabetes risk by increasing glucose uptake by muscles, improving insulin levels, and by decreasing adipose tissue. The reduction of adipose tissue could also have a positive effect on vitamin D status by reducing the amount of serum 25(OH)D which could be sequestered by the surplus of adipose tissue.

*Serum Cotinine*

In NHANES, smoking status was assessed based on the measurement of serum cotinine, which is the primary metabolite of nicotine. Smoking tobacco has been associated with T2DM by causing increases in blood sugar and has been shown to have a role in insulin secretion. Smoking has also been found to affect vitamin D status in certain subgroups of the population. Average cotinine levels for adult smokers in the US is > 100 ng/mL, however light smokers and non-smokers could have cotinine levels in the 1 to 4 ng/mL range, with higher cut-points required for non-smokers with high levels of second hand smoke exposure [i.e. living in a house with a smoker(s)]. To avoid false-positives, in this instance, subjects with serum cotinine concentrations < 10ng/mL were categorized as “non-smokers” and all others were considered smokers.
Calcium

Insulin secretion is a calcium dependent process. Vitamin D and calcium have a positive association and increased serum 25(OH)D can increase the level of calcium available for the process on insulin secretion. This association could play a role in reducing fasting insulin levels as well as glucose. The normal range for serum calcium is 8.5 to 10.2 mg/dL.

PTH

Serum PTH is a very sensitive indicator of vitamin D deficiency. When vitamin D levels are low, calcium levels also lower. In addition to maintaining strong bones, calcium is also important for many other bodily functions, such as, muscle function, carrying messages between the brain and rest of the body via the nervous system, and moving blood throughout the body.\(^27\) Therefore it is important to maintain calcium homeostasis. In order to do so, in times of low blood calcium, PTH is released to promote calcium resorption from the bones, which mobilizes the calcium found in bones back into the bloodstream. When vitamin D is low, and PTH is high, there can be a reduction in glucose uptake by target cells by reducing the number of glucose transporters (GLUT1 and GLUT4) available on the tissue membrane required for uptake.\(^5\) Normal range for serum PTH is 10.0 to 55.0 pg/mL.

Vitamin D and Body Composition

Body composition is of great relevance to the development of T2DM. It is well known that obesity is a risk factor for prediabetes and T2DM (possibly due to its chronic
state of inflammation), while muscle mass improves insulin sensitivity. Vitamin D may play a prominent role in muscle health. The clinical disorder of vitamin D deficiency is characterized by muscle weakness in proximal muscles, as well as muscle pain and impaired gait.\textsuperscript{5,28}

Many studies have investigated the link between vitamin D and muscle mass and function. Often these studies involve the elderly, to determine if sufficient vitamin D status can limit fractures by reducing falls. Several intervention studies have reported beneficial effects of vitamin D on falls. A study by Flicker et al. investigated the effects of vitamin D supplementation among 625 older elderly residents in assisted-living homes. Those who received calcium (600mg/daily) and vitamin D (10,000 IU/weekly and then 1,000 IU/daily) for 2 years were less likely to fall than those receiving calcium alone.\textsuperscript{29} Likewise, Bischoff et al. found that in 122 older women in geriatric care, calcium (1200mg/day) and vitamin D (800 IU/day) caused a 49% reduction in falls during the 12-week treatment versus calcium alone. It was also found that those who fell most had the greatest benefit from the treatment.\textsuperscript{30}

Not all studies have agreed with these results. A meta-analysis of 20 randomized, controlled trials found that supplementation with vitamin D, with or without calcium, did not reduce the risk of fall by 15% or greater, which was the risk-reduction threshold.\textsuperscript{31} Randomized, controlled vitamin D trials have often provided a mixed picture of results with some studies being positive for observed measures with others being null. In a meta-analysis by Stockton et al., vitamin D had no effect on muscle strength, unless the individuals had starting serum 25OHD levels <25nmol/l. Individuals with 25(OH)D
levels in the 25-75nmol/l range showed no improvement in muscle mass or strength with vitamin D supplementation.\textsuperscript{32}

The presence of VDR in muscle cells, reports of profound muscle weakness in children with rickets, muscle weakness as a sign of vitamin D deficiency, as well results from intervention and observational studies provides evidence of a correlation between muscle function and vitamin D, although at what level has been debated. However, any link between vitamin D and muscle could provide implications for T2DM risk. Muscle, aside from generating force, is a highly metabolic tissue that responds to a wide range of hormones, including insulin. Under normal physiological conditions, skeletal muscle is responsible for approximately 85% of whole-body insulin mediated glucose uptake.\textsuperscript{33}

**Vitamin D and the Immune System**

Inflammatory factors have often been associated with insulin resistance and reduced \( \beta \)-cell function, which are indicative of T2DM.\textsuperscript{5,7} Inflammation is part of a complex immune response against harmful stimuli such as pathogens, damaged cells, irritants, or, in the case of obesity, excess nutrients and energy.\textsuperscript{34} The immune response can be either acute, as in the case of the response to pathogens, or chronic, as is the case with obesity. A chronic state of inflammation, which is typical in cases involving T2DM, has shown to be detrimental to \( \beta \)-cells, leading to a reduction in both numbers and function of the cells, and therefore leading to a reduction of insulin secretion.\textsuperscript{37}

The presence of VDR in almost all cells in the immune system, including monocytes, macrophages, dendritic cells, and activated T and B lymphocytes has led to the investigation of vitamin D as a potential immunomodulator.\textsuperscript{6} VDR expression in
some immune cells is controlled by the presence of immune signals. For example, naïve T-cells only display very low VDR levels, however, this receptor becomes abundantly available upon T-cell activation by the immune response. In contrast, monocytes, such as macrophages and dendritic cells, which play a key role in the inflammatory response by producing pro-inflammatory cytokines such as TNF-α, IL-23, and IL-12, has been shown to be accompanied by a decrease in VDR-expression, making these cells less sensitive to vitamin D. Together, the abundance of VDR displayed throughout the immune system and their regulation by immune signals argues for an important role of vitamin D as a modulator of immune responses.

Vitamin D has been reported to have a wide range of immune actions: it promotes differentiation of monocytes to macrophages, enhancing their cytotoxic activity and antimicrobial properties; influences dendritic cell maturation, directing the dendritic cells into a more tolerogenic state with the ability to induce regulatory T cells; and modifies inflammation by altering cytokine secretion profile, inhibiting pro-inflammatory IL-12, IL-23, and TNF-α while enhancing the release of IL-10 (a cytokine which exerts broad-spectrum anti-inflammatory activities. This modulation of chemokine and cytokine secretion, which has been observed, is of particular interest in the context of diabetes due to the correlation between T2DM and inflammation.

**Vitamin D and Insulin Secretion**

A hallmark of T2DM is a reduction in insulin secretion by the pancreatic islets (β-cells). Several studies support a role of vitamin D in pancreatic β-cell function through both direct and indirect effects. The indirect effect is due first, to vitamin D having a
protective role in reducing circulating pro-inflammatory cytokines, which have been shown to cause β-cell dysfunction. Also, since insulin secretion is a calcium-dependent process and vitamin D has a well-recognized role in regulating extracellular calcium and calcium flux through β-cells, it may play an important role in the normal functioning of β-cells. \(^7,35,36\) It has been speculated that vitamin D insufficiency may alter the balance between the extracellular and intracellular β-cell calcium pools, which may interfere with normal insulin release, especially in response to a glucose load.\(^35\) Due to the importance of calcium in insulin secretion, it is important to use caution when interpreting results, since an important feature of vitamin D deficiency can be hypocalcemia, which in and of itself can dramatically affect β-cell function.

Evidence for a physiological role for vitamin D on β-cell function is supported by a high local expression of VDR by the β-cells. Thus, a direct effect of vitamin D on β-cells, and in turn, insulin secretion, may be mediated by the binding of its circulating active form 1,25(OH)\(_2\)D to the β-cell VDR. Alternatively, activation of vitamin D may occur within the β-cell by the 1-α-hydroxylase enzyme, which has been shown to be expressed in β-cells.\(^5,7,37\) The 1-α-hydroxylase gene is responsible for converting 25(OH)D into the active vitamin D metabolite, 1,25(OH)\(_2\)D.

**Vitamin D and Insulin Sensitivity**

In addition to vitamin D having a possible role in improving insulin secretion, there is evidence that vitamin D may also improve insulin resistance as well. Ashraf et al. looked at 47 female adolescents (ages 15.8 +/- 1.4 years) and found that increased levels of vitamin D binding protein were found to improve insulin resistance, as well as
fasting insulin, and whole-body insulin sensitivity. These relationships remained significant even after adjustment for percentage of body fat and race. A randomized controlled trial by Belenchia et al. looked at 35, non-diabetic, obese adolescents (ages 14.1 +/- 2.8 yrs; BMI 39.8 +/- 6.1) and randomly assigned them either placebo or 4000 IU/day of vitamin D3. After 6 months, the vitamin D group showed significant changes in fasting insulin as well as HOMA-IR, leading the authors to suggest a possible role for vitamin D in improving insulin resistance.

The effect that vitamin D has on insulin sensitivity may be through several different mechanisms. In addition to improving β-cell function by direct actions as well as protecting β-cells against immune responses with its anti-inflammatory properties, as previously mentioned, vitamin D also improves insulin sensitivity of target cells (skeletal muscle, and adipose tissue) by stimulating the expression of the insulin receptor, and enhancing insulin responsiveness for glucose transport. Studies have suggested that PTH may elicit insulin resistance by reducing the number of glucose transporters (both GLUT1 and GLUT4) available on the membrane to promote glucose uptake.

**Vitamin D and Fasting Glucose**

Observational studies looking at vitamin D’s possible relationship with fasting glucose have reported consistent associations. A cross-sectional study by Hurskainen et al., looked at 850 men and 906 women in Finland, ages 53 – 73 years, and found serum 25(OH)D to be inversely associated with fasting blood glucose. A large cohort study by Tsur et al. looked at a group of subjects, who did not have diabetes and were ages 40 – 70 years, to assess the development of impaired fasting glucose (IFG) and diabetes based
on subgroups of 25(OH)D serum levels. They found that the odds of transitioning from normoglycemia to IFG or diabetes, or from IFG to diabetes, increased in subjects with lower 25(OH)D levels. These results suggest that vitamin D deficiency appears to be an independent risk factor for the development of IFG and diabetes.

The small sample of randomized controlled trials that have investigated the possible relationship between 25(OH)D and fasting glucose have shown mixed results. A recent study randomly assigned 511 individuals with impaired fasting glucose, to either 20,000 IU/week of vitamin D or placebo. After 1-year of supplementation, there was no significant improvement in glycemic indices between the groups. It is important to note that this was the first year of an ongoing 5-year trial; therefore, a longer time period may be necessary to affect the outcomes. This study was also done in Norway and two-thirds of the subjects had baseline serum 25(OH)D levels that would be considered sufficient enough to meet the health requirements of 97.5% of the population, by the current standards used in the US. Another group looked at the effect of supplementing 1000 IU/day of vitamin D on the markers of glucose metabolism (fasting glucose, fasting insulin, A1C and HOMAIR) of 77 overweight and obese women at baseline and after 12 weeks. There were no significant differences in indices between the two groups. It is unknown whether 12 weeks is an adequate time period to achieve a beneficial response. Also, the study showed that a dose of 1000 IU/day was not adequate enough to raise the serum 25(OH)D to the target of $\geq 30$ ng/mL. Since vitamin D is sequestered in adipose tissue, a higher dose may be required.
Due to the role of vitamin D in muscle function, it is biologically plausible that there could also be a role in maintenance of blood glucose levels since muscle is the primary organ of glucose uptake.

**Vitamin D and Fasting Insulin**

The relationship between vitamin D and insulin is inconsistent. A recent study looking at 47 female adolescents (ages 15.8 ± 1.4 years, BMI 23 ± 4.0) found vitamin D binding protein to be inversely associated with fasting insulin, insulin resistance, and whole body insulin sensitivity. These relationships remained significant after adjusting for body fat percentage and race. Similarly, a dose of 4000 IU/day of vitamin D improved fasting insulin (-6.5 compared with +1.2 uU/mL for placebo; *p = 0.026*) and HOMA-IR (-1.363 compared with +0.27 for placebo; *p = 0.033*) in obese adolescents.

Using a similar dose, Nader et al. failed to show an effect of vitamin D on glucose or insulin levels in 58 obese adolescents. One possible explanation was that at baseline, both the placebo and the experimental groups had adequate serum vitamin D levels.

**Vitamin D and Glycated Hemoglobin**

While vitamin D has been shown to be inversely related to A1C in observational studies, the confounding effect of age must be addressed. Using NHANES data, vitamin D was inversely related to A1C in the 35 – 74 year age group but did not show any significance in either the 18 – 34 age group or the 75-plus age group. Confirming these results, a study by Ford et al. found there to be no significance between serum 25(OH)D and A1C in adolescents (ages 12 – 17). Another study by Manickam assessed serum
25(OH)D and A1C levels in 1074 African-American men or Caucasian men, ages 25 and older and found an inverse relationship amongst all men. Serum 25(OH)D was also found to be an independent determinant of A1C levels in African-American men, however was not in Caucasian men. Unfortunately there were no average ages given to compare the results to the previous studies mentioned, only that subjects had to be at least 25 to be eligible to participate and that the majority of subjects were under 70 years of age.

Results from a multiple logistic regression analysis from a large, nationally representative sample of adults ages 20 and older, found that serum 25(OH)D concentration had an inverse association with A1C. This result was found to be independent of a set of covariates, including, age, race, season of examination, education, lipid profiles, and behavior risk factors. A longitudinal population based multipurpose study performed by the University of Tromsø in Norway looked at both subjects with and without diabetes and investigated the correlation between serum 25(OH)D and A1C. A significant correlation between serum 25(OH)D and A1C was found in the group without diabetes and although less statistically significant than the group without diabetes, there was also found to be a correlation in the group with diabetes. Of particular note, in the group with diabetes, there was found to be a greater difference in A1C between those in the highest and lowest serum 25(OH)D quartiles, even though all of the subjects had been previously diagnosed with diabetes. The Tromsø Study found serum 25(OH)D to be significantly higher and A1C to be significantly lower in the summer months (May through August) as compared to the winter months (December through February).
Seasonal variation in serum 25(OH)D has been established and, in patients with diabetes, seasonal variation in A1C has also been observed.\textsuperscript{50}

**Vitamin D and Markers of Glucose Metabolism: Biological Plausibility**

Increasing evidence supports the hypothesis that vitamin D may play a pivotal role in the pathophysiology of glucose metabolism. To date, the exact mechanisms are not yet fully understood, however, vitamin D’s role in insulin resistance and pancreatic $\beta$-cell function may be the primary pathways in which vitamin D may impact glucose homeostasis.

Possible explanations of the biological plausibility for vitamin D’s correlation with markers of glucose metabolism are as follows:

*Vitamin D and body composition:* If vitamin D has been shown to improve muscle mass and function then it may be possible that vitamin D indirectly plays a role in glucose uptake. That is, since muscle is responsible for nearly 85% of glucose uptake from the blood, improved muscle mass and function as a result of vitamin D could increase the amount of glucose that could be taken up.

*Vitamin D and the immune system:* Vitamin D’s anti-inflammatory properties could reduce circulating inflammatory cytokines, which have been shown to be toxic to pancreatic $\beta$-cells. This results in a protective role with the $\beta$-cells, in turn increasing function as well as insulin production, which would have an inverse correlation on markers of glucose metabolism.
*Vitamin D and insulin secretion:* In addition to its protective role against inflammatory cytokines, vitamin D also regulates Ca\(^{2+}\) levels. Since insulin secretion is a calcium dependent process, vitamin D’s ability to regulate calcium flux may play a pivotal role in insulin secretion. Also, since \(\beta\)-cells have been shown to have a high expression of VDRs, sufficient serum levels of vitamin D may be important in providing enough substrate for the \(\beta\)-cells to produce insulin. A reduction in insulin secretion would have an inverse relationship on the markers of glucose metabolism, which has been discussed.

*Vitamin D and insulin sensitivity:* Similar to vitamin D’s role in insulin secretion by regulating extracellular calcium levels, vitamin D also may play a role in insulin sensitivity by regulating PTH levels. PTH, when elevated, has been shown to reduce glucose uptake by the muscle, and adipose tissue. This could have a direct relationship with markers of glucose metabolism, increasing both fasting glucose and insulin, as well as increasing the amount of glucose in the blood that could bind to red blood cells, in turn increasing A1C.

**Purpose**

The purpose of this study is to investigate the relationship between serum 25(OH)D levels and markers of glucose metabolism (i.e., A1C, fasting glucose, and fasting insulin) to determine if maintaining sufficient serum levels of vitamin D (≥30ng/mL) was related to normal values of the same.
Null Hypotheses

1. There is no relationship between serum vitamin D and markers of glucose metabolism.
2. Adequate levels of vitamin D (≥ 30 ng/mL) will not be related to abnormal levels of fasting glucose.
3. Adequate levels of vitamin D (≥ 30 ng/mL) will not be related to abnormal levels of fasting insulin.
4. Adequate levels of vitamin D (≥ 30 ng/mL) will not be related to abnormal levels of A1C.
5. The likelihood of having a diagnosis of prediabetes is not different for individuals with adequate versus inadequate levels of vitamin D (≥ 30 ng/mL)

Methods

Data Set - NHANES

Data from NHANES 2005 – 2006 were used for this thesis. This was the final NHANES data set that included serum 25(OH)D. The NHANES is conducted by the National Center for Health Statistics. Survey participants from the US noninstitutionalized, civilian population, were selected using a stratified multistage probability sample design with oversampling of certain subgroups including young Americans and Hispanics. The data are weighted to account for the complex survey design, survey nonresponse, and post stratification, and thus are considered to be generalizable for the US population. For the purpose of this study, data was accessed via the public domain.
Participants were interviewed and invited for clinical examination. Physical examination and collection of blood samples were conducted in a mobile examination clinic (MEC). Due to MEC being mobile, examinations were performed in northern latitudes in the summer months and southern latitudes in the winter months.

The NCHS Ethics Review Board approved the survey, and participants provided informed written consent.

Subjects

Adults ages 20 and over, males (n = 654) and females (n = 560), subjects who answered “yes” to the question “have you ever been told by a doctor that you have prediabetes” included (n = 48). No subjects had diabetes.

Subjects self-reported ethnicity and race. The only ethnicity categories were related to being of Hispanic, Latino, or Spanish origin. Respondents who self-identified as “Mexican American” were coded as such regardless of their other race-ethnicity identities. Otherwise, per NHANES, self-identified “Hispanic” ancestry would be coded as “other” Hispanic. The “other” Hispanic categories listed on the NHANES demographics questionnaire included Puerto Rican, Dominican, Mexican, Chicano, Cuban American, Central or South American, and other Latin American.

Subjects who were missing data for the variables and covariates needed for statistical analysis were excluded to avoid the data from being skewed.
Biochemical Measures

All serum specimens were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, CDC, using standardized measurement and analysis. Serum 25(OH)D concentration was measured using the Diasorin radioimmunoassay kit. Data from NHANES 2005 – 2006 have been adjusted for assay drifts. Serum 25(OH)D is used to estimate total intake of vitamin D from both cutaneous synthesis and dietary intake. Serum A1C was measured using a high performance liquid chromatography system. Glucose concentration was measured spectrophotometrically and insulin was measured using an insulin radioimmunoassay (RIA).

Measurement of Variables

Serum 25(OH)

Based on PTH’s role as an early indicator for vitamin D deficiency and studies that have found PTH levels plateau when serum 25(OH)D levels reach 30 – 32 ng/mL, the cut-point for serum 25(OH)D is set at 30 ng/mL. Values ≥ 30ng/mL were considered “sufficient” and values < 30ng/mL were considered “insufficient”.53

Fasting Glucose

Based on the cut points defined by the ADA, fasting glucose levels are considered normal if < 100 mg/dL, impaired (prediabetic) between 100 and 125 mg/dL, and diabetic ≥ 126 mg/dL.54
Fasting Insulin

Fasting insulin levels < 5 uU/mL are considered normal and levels ≥ 5uU/mL are considered high.

A1C

Based on ADA guidelines, A1C measurements < 5.7% are normal, levels between 5.7% - 6.4% are considered prediabetic, and ≥ 6.5% are diagnosed as diabetic. 54

Prediabetes

Prediabetes is defined as having blood sugar level, as defined by fasting glucose or A1C, that is higher than normal but not yet high enough to be diagnosed as type 2 diabetes. For this study, prediabetes was determined based on the subject answering “yes” to the question “have you ever been told by a doctor that you have prediabetes?” Subjects with prediabetes were included in the study.

Statistical Analysis

Statistical analysis was performed using statistical software (SAS Enterprise Version 6.1, SAS Institute, Cary, NC.) For this analysis, statistical significance was set at a p-value ≤ 0.05. Characteristics of the population were analyzed using independent t-tests to compare variable means for A1C, fasting glucose, fasting insulin, age, and BMI for subjects with serum vitamin D < 30 ng/mL (inadequate) and subjects with vitamin D ≥ 30 ng/mL (adequate). The relationship between vitamin D and markers of glucose metabolism (fasting glucose, fasting insulin, and A1C) were examined using Pearson’s
correlation to test the strength and direction of correlation of the variables. After initially testing for correlation, results were adjusted to control for the possible confounding variables: ethnicity (Mexican American, other Hispanic, White, African American, other race including multi-racial), age, gender, BMI, android fat mass, physical activity level, serum cotinine, serum calcium, and serum PTH. Seasonal variation was not adjusted for. The reason for not adjusting for seasonal variation is since physical exams are performed in mobile vans (MEC) for NHANES, data could not be collected in northern latitudes during the winter; instead data were collected in northern latitudes during the summer months and in southern latitudes during the winter months, thus offsetting any seasonal variation in serum 25(OH)D levels that may be otherwise observed.52 Once a relationship between vitamin D and markers of glucose metabolism was established, an odds ratio was calculated to measure whether adequate vitamin concentrations lowered the risk of abnormal markers of glucose metabolism. Serum 25(OH)D was classified as adequate if ≥ 30 ng/mL. Fasting glucose, fasting insulin, and A1C were classified based on ADA cutpoints.11 Subjects were defined as having prediabetes or diabetes if they answered yes to the following questions on a NHANES questionnaire, respectively, “Have you ever been told by a doctor that you have prediabetes?” or “Have you ever been told by a doctor that you have diabetes or sugar diabetes?” A chi-squared test was used to determine if the proportion of subjects with adequate serum vitamin D was independent of abnormal markers of glucose metabolism, as measured by A1C, fasting glucose, and fasting insulin
After assumptions were checked, one-way ANOVA was performed, to determine if there were significant differences in the mean scores of serum vitamin D, A1C, fasting glucose, and fasting insulin across ethnicities.

Results

The population sample was comprised of 47.7% Caucasian subjects, followed by 22.9% African-American and 20.9% Mexican-American subjects (Table 2).

Table 2. Self-reported Ethnicity of Sample

<table>
<thead>
<tr>
<th>*Ethnicity</th>
<th>N (1214)</th>
<th>Percentage of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexican American</td>
<td>254</td>
<td>20.9%</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>46</td>
<td>4.0%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>579</td>
<td>47.7%</td>
</tr>
<tr>
<td>African American</td>
<td>278</td>
<td>22.9%</td>
</tr>
<tr>
<td>Other Race</td>
<td>57</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

A one-way ANOVA was performed to compare serum vitamin D levels across different ethnicities. An R-squared value of 0.24 showed that 24% of the variability of serum vitamin D is explained by ethnicity. The Caucasian group had significantly higher mean levels of 25(OH)D when compared to all other ethnicities. The African-American group had significantly lower mean levels of serum 25(OH)D when compared to all other ethnicities.

Variable means were significantly different between subjects in the adequate and inadequate vitamin D categories for age, BMI, A1C, fasting glucose, and fasting insulin (Table 3).
Table 3. Characteristics of Sample (Mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin D &lt; 30 ng/mL (n=1340)</th>
<th>Vitamin D ≥ 30 ng/mL (n = 283)</th>
<th>p Value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42.22 ± 13.88</td>
<td>43.30 ± 14.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>28.56 ± 5.94</td>
<td>26.15 ± 4.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>5.36 ± 0.65</td>
<td>5.25 ± 0.48</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>99.25 ± 19.63</td>
<td>96.54 ± 14.97</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (uU/mL)</td>
<td>12.10 ± 12.69</td>
<td>7.53 ± 4.93</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\)Independent t-test; Significance set at \(p\) value ≤ 0.05

A1C

There was a small inverse correlation between serum 25(OH)D and A1C (\(r = -0.12\), \(p = <0.0001\)), which remained significant after adjusting for covariates (Table 4). The odds of having an abnormal A1C with adequate serum 25(OH)D levels was significant (OR = 0.67; CI = 0.53 – 0.99) (Table 5). Thus, those having an adequate vitamin D were less likely to have an abnormal A1C. The proportion of those in the inadequate vitamin D category was not independent of those in the abnormal A1C category (\(X^2 = 3.89\); \(p = 0.05\)). These results do not support the null hypothesis that the two variables were unrelated.

Caucasians were found to have significantly lower mean levels of A1C when compared to Mexican Americans and African Americans (Difference Between Means (95% CI) = -0.17 (-0.29 to -0.04) and -0.21 (-0.34 to -0.09), respectively).
Table 4. Correlation Between Serum Vitamin D and Markers of Glucose Metabolism in the Total Sample (N = 1214)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Pearson Correlation Coefficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1C (%)</td>
<td>-0.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>-0.08</td>
<td>0.005</td>
</tr>
<tr>
<td>Insulin (uU/mL)</td>
<td>-0.18</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Significance set at p value ≤ 0.05

**Fasting Glucose**

There was a small inverse correlation shown between 25(OH)D and fasting glucose \((r = -0.08; p = 0.005)\) (Table 4), which remained significant after adjusting for possible confounders. The odds of having impaired fasting glucose with adequate serum 25(OH)D was not significant \((OR = 0.78; CI = 0.57 – 1.05)\) (Table 5). The proportion of those in the inadequate vitamin D category were independent of those in the IFG category \((\chi^2 = 2.61; p = 0.11)\). This supports the null hypothesis that the two variables were unrelated. African Americans were found to have significantly lower mean glucose levels when compared to Mexican Americans \((p = ≤ 0.05)\) (Difference Between Means \((95\% CI) = -5.63 (-10.23 to -1.04))\).

**Fasting Insulin**

There was an inverse correlation observed between fasting insulin and serum 25(OH)D \((r = -0.18; p = <0.0001)\) (Table 4). The correlation remained after adjusting for confounders. The odds of having impaired fasting insulin with adequate 25(OH)D was found to be significant \((OR = 0.40; CI = 0.29 – 0.54)\) (Table 5). The proportion of those in the inadequate vitamin D category was not independent of those in the abnormal
fasting insulin category ($\chi^2 = 35.64; p = <0.0001$). These results do not support the null hypothesis that the variables are unrelated.

There was a significant relationship observed between fasting insulin and serum 25(OH)D among Mexican Americans and Caucasians ($\chi^2 = 19.84; p = <0.001$ and $\chi^2 = 13.33; p = 0.0003$, respectively) (Table 6). No other ethnicities showed any relationship. Caucasians were shown to have significantly lower mean insulin levels when compared to Mexican Americans (Difference Between Means = -2.77; CI = -5.25 to -0.28). No other ethnicities showed any relationship.

Table 5. Risk of Having Abnormal Markers of Glucose Metabolism With Inadequate Vitamin D Levels$^a$ in the Total Sample (N=1214)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Odds Ratio</th>
<th>95 % Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1C (%)$^b$</td>
<td>0.67</td>
<td>0.44 0.99</td>
</tr>
<tr>
<td>Glucose (mg/dL)$^c$</td>
<td>0.78</td>
<td>0.57 1.06</td>
</tr>
<tr>
<td>Insulin (uU/mL)$^d$</td>
<td>0.40</td>
<td>0.29 0.54</td>
</tr>
</tbody>
</table>

$^a$Serum Vitamin D (insufficient = < 30 ng/mL; adequate = $\geq$ 30 ng/mL)
$^b$A1C (normal = < 5.7%; elevated = $\geq$ 5.7%)
$^c$Fasting glucose (normal = < 100 mg/dL; elevated = $\geq$ 100 mg/dL)
$^d$Fasting insulin (normal = < 5 uU/mL; elevated = $\geq$ 5 uU/mL)

Significance set at $p \leq 0.05$

**Prediabetes**

The odds of having prediabetes with adequate 25(OH)D was not significant (OR = 1.65; CI = 0.70 – 3.90). Those in the inadequate vitamin D category were independent of those who answered yes to being diagnosed with prediabetes ($\chi^2 = 2.38; p = 0.30$). These results support the null hypothesis.
Table 6. Risk of Having Abnormal Markers of Glucose Metabolism With Inadequate Vitamin D Levels<sup>a</sup> by Ethnicity

<table>
<thead>
<tr>
<th>Marker</th>
<th>N</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mexican American</td>
<td>254</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C</td>
<td>1.33</td>
<td>0.50</td>
<td>3.51</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.71</td>
<td>0.31</td>
<td>1.62</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.16</td>
<td>0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C</td>
<td>3.80</td>
<td>0.29</td>
<td>49.91</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.38</td>
<td>0.28</td>
<td>40.25</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.32</td>
<td>0.02</td>
<td>4.16</td>
</tr>
<tr>
<td>Caucasian</td>
<td>579</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C</td>
<td>0.88</td>
<td>0.51</td>
<td>1.53</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.72</td>
<td>0.50</td>
<td>1.05</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.50</td>
<td>0.34</td>
<td>0.73</td>
</tr>
<tr>
<td>African American</td>
<td>278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C</td>
<td>2.14</td>
<td>0.52</td>
<td>8.76</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.45</td>
<td>0.81</td>
<td>14.75</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.82</td>
<td>0.16</td>
<td>4.17</td>
</tr>
<tr>
<td>Other</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C</td>
<td>0.29</td>
<td>0.03</td>
<td>2.53</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.49</td>
<td>0.11</td>
<td>2.12</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.63</td>
<td>0.14</td>
<td>2.89</td>
</tr>
</tbody>
</table>

<sup>a</sup>Serum Vitamin D (insufficient = < 30 ng/mL; adequate = ≥ 30 ng/mL)
<sup>b</sup>A1C (normal = 5.7%; impaired = ≥ 5.7%)
<sup>c</sup>Fasting glucose (normal = < 100 mg/dL; elevated = ≥ 100 mg/dL)
<sup>d</sup>Fasting insulin (normal = < 5 uU/mL; elevated = ≥ 5 uU/mL)

Significance set at <i>p</i> ≤ 0.05

Discussion

Using data from a large, nationally representative sample of adolescents and adults age 20 to 70, the findings of this study showed serum vitamin D to be significantly inversely correlated with the markers of glucose metabolism; A1C, fasting glucose, and fasting insulin. There are few studies that have investigated the correlation between serum vitamin D and multiple markers of glucose metabolism. Instead, current studies tend to focus on outcomes that often involve a single marker of interest. Recent studies
also often focus on specific populations, such as obese adolescents, or the elderly, and often have small population sizes, where this study is multi-ethnic, includes adults ages 20 – 70 and has a large population size of 1214, making comparisons to other available studies challenging.

The relationship between serum 25(OH)D and A1C found in this study is consistent with the findings from the Tromsø Study, which found serum vitamin D levels to be inversely associated with A1C. The Tromsø Study, however, was done in a predominately Caucasian population in Norway and these findings were not a reflection of the multi-ethnic population used for this study. Our results were mixed compared to the findings by Manickam et al. which found 25(OH)D and A1C to be inversely associated in all men and also found serum 25(OH)D levels to be an independent determinant of A1C in African-American men, but not in Caucasian men. In this study, there was shown to be a small inverse correlation between A1C and 25(OH)D and inadequate 25(OH)D levels were found to be associated with raised A1C levels.

Where our results deviated from that of the Manickam study was, even though this study found African Americans to have statistically significant higher levels of A1C, when compared to Caucasians, insufficient levels of 25(OH)D were not found to be associated with increased A1C levels in neither African-American nor Caucasian men. The Manickam study was done in a population that included men, ages 50 – 70 and took place at an urban Veterans Administration Medical Center in Washington DC. Aside from the study including age groups that have a higher risk for impaired markers of glucose metabolism and T2DM, (risk increases at age 45) the fact that the subjects were recruited while receiving outpatient services at a medical center could indicate that the
subjects are receiving treatment for illness or ailments which could have an effect on either vitamin D status or their markers of glucose metabolism, or both. This could be justification for the difference in results when compared to this study. Two studies by Kositsawat, also found there to be an inverse relationship between 25(OH)D and A1C, which are supported by the findings from this study.\textsuperscript{45,56} The most recent of the Kositsawat studies found that vitamin D insufficiency, was associated with an increased likelihood of having abnormal A1C levels, which are confirmed by the findings of our study that showed adequate levels of 25(OH)D was associated with a decreased likelihood of having abnormal A1C.\textsuperscript{59} The Kositsawat study, however, focused on an elderly population (ages 70 – 79 years), while our ages ranged from 20 to 70, and Kositsawat also used 20 ng/mL of serum 25(OH)D as their cut-point to be considered insufficient/sufficient, compared with the 30 ng/mL used for this study.

The results of this study agreed with an observational study by Hurskainen, which looked at a large cohort of men and women in Finland and found there to be an inverse correlation between 25(OH)D and fasting glucose.\textsuperscript{40} The Hurskainen study was performed with a large population group of 850 men and 906 women, however, demographics for Finland show approximately 98% of the population to be Caucasian, which is not representative of the multi-ethnic population which was utilized for this study. Hurskainen also used 20 ng/ml as the cut-point to be considered vitamin D sufficient. The lower standard for serum vitamin D levels to be considered sufficient do not compare to the 30 ng/ml set by this study, and therefore may have led to results showing a larger relationship between serum 25(OH)D and fasting glucose than what the results of this study indicated. The results of this study confirmed the results of two
intervention studies that investigated the relationship of serum 25(OH)D with fasting glucose. Sollid et al. administered 20,000 IU of vitamin D per week to a group of subjects that had been diagnosed with prediabetes and found no improvement in fasting glucose levels, compared to the placebo group. These results were reported at the 1-year mark of an ongoing 5-year study involving 511 subjects with IFG or impaired glucose tolerance. A study by Salehpour et al. investigated the effects of 1000 IU/day over 12 weeks, when given to a group of 77 healthy overweight and obese women (BMI 29.9 ± 4.2 kg/m²). The resulting data showed no difference between the vitamin D group and the placebo group, indicating vitamin D had no significant effect on fasting glucose levels, among overweight and obese women. Although the results of this study agree with those found by Salehpour, the population studied by Salehpour differed greatly from that of this study. Given that Salehpour’s subjects were obese, the effect of 1000 IU/day on vitamin D status may not be great enough to significantly increase vitamin D status to reach the levels needed to provide significant results. Due to possible sequestration of vitamin D by adipose tissue, obese individuals may require higher levels of vitamin D to achieve the levels used to be considered sufficient in this study.

A large cohort study by Tsur found that the odds of transitioning from normoglycemic to either impaired fasting glucose or diabetes, increased in subjects with lower serum 25(OH)D levels compared to those with higher levels. Tsur used five subgroups of vitamin D status, with the reference group being 75 nmol/L, which is approximately the same as the cut-point established in this study to determine sufficient levels of 25(OH)D (30 ng/mL). Our results do not show that sufficient levels of 25(OH)D are associated with normal fasting glucose, therefore not supporting the results
found by Tsur. The Tsur study was performed in Israel and the study population was non-diabetic subjects ages 40 – 70. There are many variables that could account for the differences in results, including differences in diet, climate, geography, and a median age range that is approximately 20 years higher than that of our population. All of these variables could have an impact on serum vitamin D.

Possible reasons for the non-significant results found in this study in relation to fasting glucose could be because of the rates of hyperinsulinemia, which was found amongst the subjects. Based on results found in relation to insulin, the high insulin levels shown could be compensating for fasting glucose levels that could be indicative of IFG, in turn lowering the observed fasting glucose levels.

In this study, serum 25(OH)D was found to be significantly inversely correlated to fasting insulin. Our findings are consistent with previous epidemiological studies that suggest an inverse association between 25(OH)D and insulin resistance. These results support the findings of Ashraf et al., which found vitamin D binding protein to be inversely associated with both fasting insulin and insulin resistance in a group of 47 adolescent females. Ashraf’s population of adolescent girls is different than the population used for this study. Investigating insulin levels among adolescents should be done with caution due to the hormonal changes associated with puberty and the effects that they have on increasing insulin resistance among this age group. Hurskainen also found an inverse relationship between serum 25(OH)D and fasting insulin, which are in support of our findings (the contrasts between this study and the Hurskainen study were described in detail previously). These results were also confirmed by a double blind study by Belenchia et al., which found significant improvements in fasting insulin in a
treatment group of obese adolescents supplemented with 4000 IU/day of vitamin D compared to the controls. There was not, however, found to be any significant change in fasting glucose in the treatment group compared to controls, which were confirmed by the results of this study. The study population used in the Belenchia study differed from that of this study in that it was comprised only of obese adolescents (ages 14.1 ± 2.8 years, BMI 39.8 ± 6.1) and only consisted of Caucasians and African Americans.

The results from our study disagreed with the results from a double blind study by Nader et al, which also included obese adolescents. Nader found that 2000 IU/day administered to the treatment group for 12 weeks led to an increase in serum 25(OH)D, however did not show significant improvements in either fasting insulin or fasting glucose. Both the Nader and Belenchia studies, aside from including only obese adolescents, also were done with a small “n” (compared to our “n” of 1214), which may be considered a limitation of these studies. With the growing concern for diabetes risk among adolescents, there inevitably is going to be an increased need for preventative measures to coincide with exercise and diet recommendations in this population. This may be the justification for the multitude of studies investigating vitamin D’s relationship with markers of glucose metabolism, and therefore diabetes risk, among adolescents.

The results of this study disagreed with the results found by Scragg et al. The Scragg study found serum 25(OH)D to be inversely associated with fasting glucose among Mexican Americans ($p \leq 0.05$) (which our study did not confirm) and also stated that the relationship between 25(OH)D and fasting insulin among non-Hispanic whites just failed to reach significance. The results of our study did not find 25(OH)D to be associated with fasting glucose among Mexican Americans and the results indicate that
there is in fact a significant relationship between 25(OH)D and insulin among both Caucasians and Mexican Americans. Where this study and the Scagg study did agree was in relation to the results for Mexican Americans and insulin. These results pertaining to Mexican Americans are a unique finding and require attention. Since it used NHANES data, the Scagg study had a similar population to this study, however had a much larger “n” compared to this study (n = 6,228 and n = 1214, respectively), presumably due to Scagg using a data set that comprised of 6 years. One difference between the Scagg study and this study that requires mentioning is the time period in which the data was collected. In the last 20 years there have been changes that could have an influence on both vitamin D status and glucose metabolism. First, according to CDC data, the rise in obesity between 1988 and 2008 increased almost 70%, with 22% of the population being obese in 1988 and approx. 32% being obese in 2008. 61 This rise in obesity could play a role both in lower vitamin D status and an increase in impaired glucose metabolism, based on mechanisms described previously. Second, with the advent of technological advances since the time period of the Scagg data, it is possible that outdoor activities, which could lead to an increase in vitamin D status, have been replaced by computers, televisions, and video games. 62,63

The lack of significant results found for serum 25(OH)D and prediabetes could be indicative of the lack of diagnosis that is often observed. The criteria used for prediabetes in this study was that the subjects had previously been told by a doctor that they had prediabetes. The number of subjects who had been told that they had prediabetes in the study population was 48. Given that statistically, 1 out of every 3 people in the US have markers of glucose metabolism that are associated with
prediabetes, it would be expected that approximately 400 subjects in this study could have prediabetes. Since markers of glucose metabolism were not used as the criteria for prediabetes in this study, there could be lack of significance shown in the results.

The strength of this study is that we analyzed data from NHANES, which is a large, multi-ethnic, nationally representative data set that oversampled certain population subgroups of public health interest. The main limitation of our present study is that findings are driven from a study with cross-sectional design. Therefore, all results cannot be interpreted as a cause and effect association. Also, there were no direct measures of insulin resistance and β-cell function used, such as HOMA-IR or C-peptide. Use of these markers could have further established a link between vitamin D and insulin resistance.

**Conclusion**

The results of this study show an inverse association between serum vitamin D and A1C, fasting glucose, and fasting insulin. Adequate levels of serum vitamin D (≥ 30 ng/mL) was also found to be associated with reduced risk of impaired fasting insulin among Mexican-American and Caucasian ethnicities. This data extends previous research and provides new evidence that serum vitamin D may play a role in impaired markers of glucose metabolism, which may have implications for the pathogenesis of type 2 diabetes. Future studies are needed to further evaluate the relationship between vitamin D and markers of glucose metabolism, especially among Mexican Americans and Caucasians.

References


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