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Treatment of Vitamin D Deficiency in Special Conditions:
Hypertension and Cystic Fibrosis

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a dissertation submitted to the Faculty of the Graduate
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of the requirements for the degree of
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Abstract

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Vitamin D deficiency is common and under treated in the United States because the disease is silent and there are limited evidence based regimens for treatment. Assessing vitamin D status and aggressively treating vitamin D deficiency in model disease populations who are at high risk for vitamin D deficiency could help define a treatment strategy for the population at large. Individuals with hypertension and cystic fibrosis (CF) are model populations since both groups are at risk for vitamin D deficiency. The goals for this thesis project are to define the clinical scope of vitamin D deficiency in individuals with hypertension and CF and to determine the appropriate population specific treatment strategy to restore vitamin D status. We first examined the prevalence of vitamin D deficiency in individuals with CF and hypertension and found both populations have higher rates of vitamin D deficiency compared with non-diseased controls. Further, we found that vitamin D deficiency was sub-optimally treated in both populations. We then examined different strategies to correct vitamin D status in both populations. In the CF population, we examined 600,000 IU of both vitamin D_2 and vitamin D_3 and UV light and found vitamin D_3 to be superior at raising 25-hydroxyvitamin D (25(OH)D), the best marker for vitamin D status. In hypertensive individuals, we examined in a pilot study the effect of calcitriol, the active form of

vitamin D, vitamin D_3 and placebo on blood pressure, a process thought to be regulated by vitamin D. Although vitamin D_3 corrected vitamin D status the best in the three groups, we found that only calcitriol therapy significantly reduced blood pressure. Vitamin D deficiency is common and can be corrected with adequate vitamin D supplementation. The role of vitamin D in hypertension in humans still needs to be clarified in terms of the correct form and dose of vitamin D to lower blood pressure and the mechanism for vitamin D's anti-hypetensive action.

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CHAPTER 1: INTRODUCTION

Vitamin D deficiency (defined as 25(OH)D <20 ng/mL) is widespread in the United States (1, 2). In the past thirty years, the number of articles published on vitamin D has quadrupled indicating an explosion in the science and understanding of vitamin D (3). There are a host of conditions precipitated by and/or exacerbated by vitamin D deficiency such as osteoporosis and rheumatoid arthritis (4-7). Recent evidence has demonstrated the importance of vitamin D in maintaining optimal immune function (8) and preventing cancer (9). In fact, resolution of deficiency may provide inexpensive prophylaxis for many conditions. However, treatment of vitamin D deficiency is largely overlooked in clinical practice (10).

Lack of awareness and agreement in the medical community regarding the burden of vitamin D deficiency, definition of vitamin D deficiency, and regimens for treatment of vitamin D deficiency are at the root of the problem (11). In part, this is because vitamin D requirements may vary by disease and even by person which complicates the development of a treatment protocol. Further, certain populations may be particularly sensitive to the consequences of low circulating 25(OH)D levels (12). Closer examination of the benefit of sufficient vitamin D nutriture using model populations provides the framework for treatment of this condition in general. Two such populations are individuals with hypertension and cystic fibrosis (CF).

Hypertension and cystic fibrosis (CF) are very different diseases; however, individuals with these conditions share a higher risk for developing vitamin D deficiency compared with non-diseased controls (13-16). CF is a rare genetic condition with the

root cause of vitamin D deficiency attributed to fat malabsorption and decreased sun exposure related to decreased outdoor activity (17, 18). In CF, vitamin D deficiency is associated with low bone density, decreased lung function, and immune function (19). Therefore, the CF Foundation has recommended actively treating vitamin D deficiency.

Conversely, hypertension is a common chronic condition affecting nearly one third of the US population (20). The cause of the vitamin D deficiency is largely unknown but may be due to decreased dietary intake, decreased sun exposure, and excessive excretion of the vitamin D carrier protein, vitamin D binding protein (DBP) (2, 21). Currently there is no consensus medical opinion regarding treatment of vitamin D deficiency in hypertensives, even though low vitamin D levels are associated with increased blood pressure, diabetes, and cardiovascular disease (22-25).

The *goals for this thesis project* are 1) to define the clinical scope of vitamin D deficiency in individuals with hypertension and CF, 2) to determine the appropriate population specific dosing strategy to restore 25(OH)D levels, and 3) to examine phenotypic changes resulting from correction of vitamin D deficiency. Several protocols have been published to correct vitamin D status (13, 26-29). However, there are conflicting reports on the effectiveness of various treatment strategies (30). Further, there is no universally accepted method for vitamin D repletion and some are concerned about toxicity (31). Therefore, a primary focus of this dissertation was to examine the efficacy and safety of vitamin D repletion strategies in both the cystic fibrosis and hypertensive patient populations. The two objectives for this aim was to determine the form and dosage of vitamin D that would result in optimal vitamin D levels without causing vitamin D toxicity.

The *central hypothesis* for this body of work is that vitamin D deficiency is common and untreated in hypertensives and CF patients and alternative treatment strategies are necessary to improve health in these populations. There are four sub-hypotheses that were tested throughout the course of this thesis project.

The *first* hypothesis is that vitamin D deficiency is associated with increased blood pressure in both blacks and whites. The justification for this hypothesis is found in epidemiological studies in which low 25(OH)D levels were associated with incident hypertension and heart disease.

The *second* hypothesis is that vitamin D therapy (active and/or vitamin D_3) will lower blood pressure in blacks with uncontrolled hypertension. This hypothesis is based on clinical trial data in which vitamin D_3 improved blood pressure control in elderly women and by animal data demonstrating blood pressure reduction is mediated through decreasing the signaling of the renin endocrine system.

The *third* hypothesis is that vitamin D deficiency is common and untreated among individuals with cystic fibrosis (CF) despite current recommendations regarding correction of vitamin D deficiency. This hypothesis is based on data from the CF foundation and clinical and epidemiological studies demonstrating inadequate levels of 25(OH)D in CF patients.

The *fourth* hypothesis is that current treatment strategies for correction of vitamin D deficiency in CF patients are not effective and that alternatives such as vitamin D_3 and/or UV lamp therapy will be superior at correcting vitamin D deficiency. This hypothesis is based on recent clinical studies demonstrating the ineffectiveness of vitamin D_2 at increasing 25(OH)D levels.

Specific Aim #1

Examine the association of blood pressure with 25(OH)D in blacks and whites in the United States using NHANES data. The approach to this aim was to use linear regression to explore this relationship. Previous studies have been done in mainly white populations and it is not known if similar associations would be seen in a broader U.S. population. The hypothesis for this aim was that blacks would have a higher prevalence of vitamin D deficiency and hypertension and the two conditions would be more strongly associated than in whites.

Specific Aim #2

Determine the most effective strategy for correcting vitamin D deficiency in adults. The approach to this aim was to examine vitamin D treatment regimens retrospectively using medical records at the Veterans Affairs Medical Center (VAMC) in Atlanta, GA. The goal of this aim was to determine the amount of vitamin D necessary to correct vitamin D status and to examine in a pilot study the determined dose of vitamin D_3 that would quickly and effectively raise 25(OH)D levels to optimal levels (> 30 ng/mL). This dose was then used in subsequent aims to evaluate the effect of correction of vitamin D status in vitamin D deficient individuals.

Specific Aim #3

Determine the effect of different forms of vitamin D in vitamin D deficient, hypertensive individuals on 24 hour ambulatory blood pressure. The approach to this aim was a three arm randomized, single blinded, clinical trial using patients with uncontrolled hypertension. We determined blood pressure by 24 hour ambulatory blood

pressure measurements before and after one of three treatment regimens (placebo, vitamin D_3 or $1,25(OH)_2D$).

Specific Aim #4

Determine if 25(OH)D levels are being maintained within those suggested by the Cystic Fibrosis Foundation. To address this aim, we evaluated vitamin D status in individuals seen at the Emory Cystic Fibrosis Center retrospectively over a two year period, 2005-2006. We further correlated markers of bone health and respiratory health to circulating 25(OH)D levels.

Specific Aim #5

Determine the most effective strategy for correcting vitamin D deficiency in CF patients using vitamins D_2, D_3, or UV light. The approach to address this aim was a randomized clinical trial of adult CF patients at the Emory CF center. Subjects were randomized to one of three arms: vitamin D_2, D_3, or UV light. We examined vitamin D status by measuring all forms of circulating 25(OH)D (25(OH) D_2 and 25(OH) D_3) to determine the overall and specific contribution of each treatment to circulating 25(OH)D levels.

CHAPTER 2 LITERATURE REVIEW

Vitamin D: History, Structure, and Function

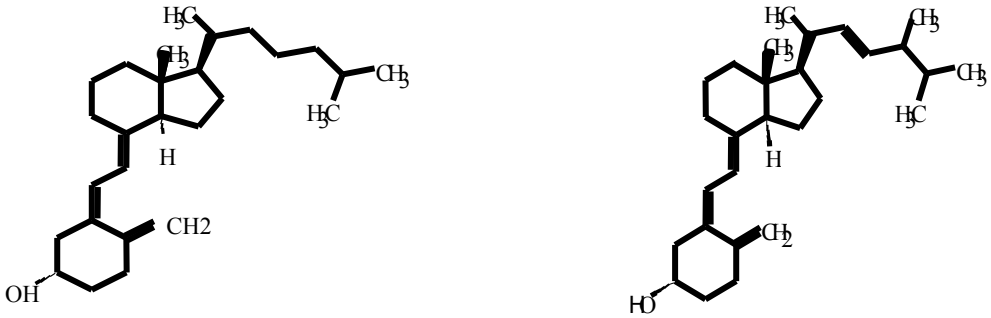
History

Vitamin D became a nutrient of note when, at the turn of the twentieth century, Sir Edward Mellanby established that cod liver oil had anti-rachitic activity (32). However, it was McCollum who later determined that a nutrient present in cod liver oil was responsible for its anti-rachitic activity and named this nutrient vitamin D (33). Mellanby originally suggested vitamin A in cod liver oil prevented rickets; however, McCollum concluded this was not the case. He destroyed the vitamin A in the cod liver oil by heating it and the oil still had anti-rachitic activity. Thus, he declared the new substance remaining in cod liver oil to be vitamin D (34). However, even before the discovery that cod liver oil could cure rickets, Trousseau of France and Palm of Great Britain noted that sunlight could be used to cure rickets, though it was not known that this too was through the vitamin D endocrine system (35). It is now known that vitamin D is present in limited food such as cod liver oil and produced endogenously in the skin.

Forms and structure

Vitamin D is a secosteroid differing from other steroids like estrogen and cortisol in that one of the rings is broken (36). Vitamin D can be obtained in the diet and from *de novo* synthesis in the skin (34). Two main forms of vitamin D exist: cholecalciferol

(vitamin D_3) and ergocalciferol (vitamin D_2) (Figure 2.1). Ultraviolet B (UVB) light converts 7-dehydrocholesterol in the skin to vitamin D_3 (37). Dietary sources of vitamin D include fatty fish, dietary supplements, and fortified milk; however, the majority of circulating vitamin D comes from sunlight exposure through the manufacture of cholecalciferol (vitamin D_3) (38-40). Ergocalciferol (vitamin D_2) is found only in supplements, fortified foods and in the diet from irradiated fungi used to fortify foods and can not be made in the skin (41).



Vitamin D_3
(cholecalciferol)

Vitamin D_2
(ergocalciferol)

Figure 2.1 Forms of Vitamin D

Physiology

Both vitamin D_3 and D_2 are acted upon by 25-hydroxylase in the liver and converted to 25(OH)D by a cytochrome P450 enzyme located in the mitochondria or a microsomal enzyme in the cytoplasma (42). Recent studies suggest that vitamin D_3 is more effective than vitamin D_2 at increasing 25(OH)D levels; (43-45) however, one study suggests this may be a dose and time dependent phenomenon (30). Vitamin D and

25(OH)D circulate in the body bound to vitamin D binding protein (DBP), albumin, and to a lesser extent unbound (46, 47). Vitamin D binding protein, also known as Gc-globulin, is a 52,000 Dalton protein and transports all forms of vitamin D but has the highest affinity for 25(OH)D (37). Vitamin D binding protein is filtered by the glomerulus in a similar manner to albumin and is reabsorbed by a megalin dependent receptor in the proximal tubule of the kidney (48).

After twenty-five hydroxylation in the liver, 25-hydroxyvitamin D is converted by 1α-hydroxylase in the kidneys to form the active steroid hormone, calcitriol or $1,25(OH)_2D$ (49). The twenty-five hydroxylation process is relatively unregulated and dependent upon the amount of substrate (D_2 or D_3), but the 1-alpha hydroxylation is tightly regulated (42, 43). Production of $1\alpha,25(OH)_2D$ is up-regulated by parathyroid hormone, therefore dependent upon calcium and phosphate status, and down-regulated by $1\alpha,25(OH)_2D$ levels (3, 11). 25(OH)D can also be 24-hydroxylated and excreted from the body (42).

Genomic functions

The active form of vitamin D, $1\alpha,25(OH)_2D$, has many functions including signal transduction and gene transcription (3). $1\alpha,25(OH)_2D$ binds to its receptor, the vitamin D receptor (VDR), and enters the nucleus where it enables transcription of those genes with a vitamin D response element (VDRE) (12). The VDR has a high affinity for $1\alpha,25(OH)_2D$ as opposed to other forms of vitamin D. It has been estimated that the VDR regulates the expression of ~2.5% of the genes in the human genome (50). The

genes range in function from bone metabolism, to cell differentiation and proliferation, (3).

Non-genomic functions

In addition to cellular regulation at the nuclear transcription level, 1α,25$(OH)_2$D also has rapid actions (51). Huhtakangas et al. have shown that the VDR present on cell membranes is associated with caveolae involved in signal transduction (52). Activation of the VDR on the cell membrane can result in a variety of signal transduction responses including second messenger systems such as protein kinase C and phosphatidylinositol-3-kinase (PI3K) (53-55). Any number of cellular functions could be regulated in this way. The fact that 1α,25$(OH)_2$D could have both rapid and genomic responses helps to explain some of the differences between the acute responses to 1α,25$(OH)_2$D and longer term responses outlined later in the chapter.

Assessing and Treating Vitamin D Deficiency

Adequate vitamin D status

Since 1α,25$(OH)_2$D has a very short half life, 25(OH)D is the form used to establish a vitamin D status (37). The concentration of 25(OH)D is measured in either ng/mL or according to SI units, nmol/L (there are 2.5 nmol/L for each 1 ng/mL) Historically, a person was considered to be deficient in vitamin D if 25(OH)D levels were less than 20 ng/mL or 50 nmol/L (56). These levels were defined based on circulating

25(OH)D levels in the U.S. population that appeared to be adequate in preventing bone disease.

Some have argued for higher circulating 25(OH)D levels to go beyond simple prevention of rickets and osteoporosis (Table 2.1) (11, 57). Recent studies suggest the relationship between parathyroid hormone and 25(OH)D does not reach its nadir until 25(OH)D levels reach 75-80 nmol/mL or 30-32 ng/mL (58). Therefore, most experts believe that vitamin D insufficiency should be defined as serum levels less than 30 ng/mL or 75 nmol/mL.

Table 2.1 Serum 25-Hydroxyvitamin D [25(OH)D] Concentrations and Health

(adapted from the Office of Dietary Supplements Dietary Fact Sheet)

ng/mL	nmol/L	Health status
<11	<27.5	Associated with vitamin D deficiency and rickets in infants and young children
<10-15	<25-37.5	Generally considered inadequate for bone and overall health in healthy individuals
≥30	≥75	Proposed by some as desirable for overall health and disease prevention, although a recent government-sponsored expert panel concluded that insufficient data are available to support these higher levels
Consistently >200	Consistently >500	Considered potentially toxic, leading to hypercalcemia and hyperphosphatemia, although

human data are limited

Vitamin D intake

As mentioned above, there is very little vitamin D in food. In fact, the primary way to obtain vitamin D is from exposure to sunlight. As such some individuals are more at risk for deficiency than others. In particular, older individuals and those with darker skin are a greater risk for deficiency due to decreased production of vitamin D in the skin (38). Since the primary dietary source of vitamin D is in fatty fish, which is not a heavily consumed item in the American diet, the majority of the United States consumption of vitamin D comes from fortified foods (Table 2.2).

Table 2.2 Amount of Vitamin D in Food

(adapted from the Office of Dietary Supplements Dietary Fact Sheet)

Food	IUs per serving*
Cod liver oil, 1 tablespoon	1,360
Salmon, cooked, 3.5 ounces	360
Mackerel, cooked, 3.5 ounces	345
Tuna fish, canned in oil, 3 ounces	200
Sardines, canned in oil, drained, 1.75 ounces	250
Milk, vitamin D-fortified, 1 cup	98

Fortified Cereal	40*
Egg, 1 whole (vitamin D is found in yolk)	20
Liver, beef, cooked, 3.5 ounces	15
Cheese, Swiss, 1 ounce	12

* Amount may vary

Correction of vitamin D deficiency

Several treatment strategies exist for restoring vitamin D levels to sufficient status (11). An individual can spend more time in the sunshine or ingest larger quantities of vitamin D (38). The amount of vitamin D needed depends on the initial 25(OH)D level (59, 60). In healthy individuals serum 25(OH)D rises 1 ng/mL for every 100 IU of additional vitamin D each day (61). Multivitamins contain between 400 IU of vitamin D which is estimated to increase 25(OH)D by 2.8 to 4.8 ng/mL which will not restore vitamin D status in a person who is vitamin D deficient (57). Another strategy for the individual with vitamin D deficiency is treatment with vitamin D 50,000 IU per week for eight weeks (62). Correction of vitamin D deficiency depends on the severity of disease and could be achieved by both sunlight and oral therapy (11).

Co-Morbidities Associated with Vitamin D Deficiency

Hypertension and cystic fibrosis (CF) are very different diseases; however, individuals with these conditions are more likely to have vitamin D deficiency than

healthy controls (13-16). Even in otherwise healthy individuals, vitamin D deficiency has been associated with disease of the heart, bones, endocrine, and immune systems (12).

Osteoporosis

Vitamin D has been studied extensively in the context of osteoporosis (63). Osteoporosis is a skeletal condition resulting from decreased bone strength and increased risk of fracture. A recent meta analysis examined 17 trials that contained 52,625 individuals and found vitamin D treatment of at least 800 IU to be beneficial in preventing fractures (28). Further vitamin D supplementation has been shown to prevent falls in the elderly which further reduces complications from osteoporosis and also suggests a role for vitamin D in maintaining muscle health or neurological balance (64). Further, hypertension is associated with an elevated risk of osteoporosis and vitamin D deficiency (65, 66). Due to increased risk of vitamin D deficiency, cystic fibrosis patients often develop metabolic bone disease as they are living longer into adulthood (19, 67, 68).

Diabetes

Vitamin D has been examined in association with both Type I and Type II diabetes (3, 12, 23, 69-72). Type I diabetes risk can be reduced by aggressive treatment with vitamin D early in life (12). Cross-sectional studies have demonstrated an association between hyperglycemia and hypovitaminosis D (69, 72). In a longitudinal study of Finnish men and women, a 40% reduction in risk of developing Type II diabetes

was observed after 17 years of follow-up (71). Further, the vitamin D receptor has been identified in the human pancreas providing molecular evidence that vitamin D is involved in pancreatic cell function and insulin regulation (73, 74). In addition to being associated with increased risk of diabetes, low vitamin D levels have been associated with increased risk of complications associated with diabetes such as all cause mortality, myocardial infarction and cardiovascular disease (22, 24).

Inflammation

Vitamin D has been identified as a potent modulator of inflammation in vitro by preventing maturation of dendritic cells (75) and by reducing inflammatory profile of T cells (76). Reduction in inflammation is achieved by a reduction in TNF-alpha and other inflammatory molecules (cytokines) associated with immune regulation (77, 78). Extending beyond the bench, vitamin D_3 therapy was found to be associated with a reduction in inflammatory profile in individuals with congestive heart failure (79).

Disease Specific Considerations: Cystic Fibrosis

CF is one of the most fatal genetic diseases affecting mainly non-hispanic whites (18). CF was first described in 1938 when infants dying of malnutrition were found to have mucous blocking the ducts of the pancreas (80). It is now known that CF is an autosomal recessive disorder characterized by chronic respiratory failure (the most common cause of death), recurrent infections and pancreatic insufficiency, leading to

decreased life expectancy (18). However, due to advances in care including pancreatic enzyme therapy and anti-biotic therapy, life expectancy has improved dramatically.

Complications of disease

In the early part of the twentieth century children diagnosed with CF rarely lived to attend school and often died of malnutrition (80). The Cystic Fibrosis Foundation was founded in 1955 to identify a cure and increase life expectancy (18). By 1985 life expectancy increased to the mid-twenties and currently the median survival age is now 36.5 years.

The benefit of increased years of life is paired with an increase in other health considerations such as CF-related diabetes and osteoporosis (19, 81). As has been suggested above, both of these co-morbidities may be exacerbated by vitamin D deficiency (63, 76). Treatment of vitamin D deficiency may be complicated in CF patients due to malabsoprtion of fat and fat soluble vitamins (17). Therefore correction of vitamin D deficiency is not as straight forward as in other populations. In fact it has been shown that even with routine supplementation, vitamin D levels are not sufficient in CF patients (13, 82).

Prevalence of vitamin D deficiency and insufficiency

The prevalence of vitamin D insufficiency in patients with CF (CF) has been reported to be as high as 90% (15, 82). This is significantly higher than healthy controls from a similar geographic area (15) or when compared to 25-hydroxyvitamin D [25(OH)D] levels from the Third National Health and Nutrition Examination Survey

(NHANES III) (83). The CF consensus statement recommends that vitamin D supplementation should be given to all patients with CF; however, routine use of vitamin D supplementation in CF patients has not resulted in increased levels (82, 84). Poor absorption of fat soluble vitamins due to pancreatic insufficiency and diminished exposure to sunlight are thought to be among the causes for low vitamin D levels in this population (17).

Management of vitamin D deficiency

Individuals with CF require routine visits to a specialized CF center for long term medical care. Key clinical concerns are lung function, infection control, skeletal health, and nutritional health. Current guidelines by the CF Foundation Consensus Conference for vitamin D supplementation with high dose vitamin D_2 appear to be inadequate (82). As was suggested above in the healthy population vitamin D_3 may be a better choice for correcting vitamin D deficiency (85, 86). An alternative to oral vitamin D supplementation in CF patients is UV light therapy to produce vitamin D in the skin. There are two ways to administer UV light. An individual can be counseled to increase their sunlight exposure or can be advised to use a lamp that emits UVB light for a prescribed amount of time. A portable table-top UV lamp can provide the UVB radiation that sunlight cannot provide efficiently in northern latitudes during the winter months (68). Although UV light therapy avoids the problem of fat malabsorption and has the potential to be therapeutic (17, 87), it is more time consuming than using a pill and therefore compliance may be an issue.

Disease Specific Considerations: Hypertension

Several lines of evidence suggest circulating vitamin D levels, both $1,25(OH)_2D$ and 25(OH)D, may be associated with blood pressure. Wild type with depleted $1,25(OH)_2D$ go on to develop hypertension (88). This increase in blood pressure can be reversed upon treatment with $1,25(OH)_2D$ and captopril, an angiotensin converting enzyme (ACE) inhibitor. Ecological studies have reported that residents of higher latitudes experience decreased UV light exposure, reduced vitamin D production, and higher systolic and diastolic blood pressure (89). Further, blood pressure is known to increase in the winter in the northern hemisphere when vitamin D production is very low (90). Prospective epidemiological studies basing vitamin D status on 25(OH)D and not dietary intake support this finding as well (91-96). In previous epidemiological studies, blood pressure was not associated with reported dietary vitamin D intake (94, 95, 97); however, those investigations did not include 25(OH)D determinations. When circulating 25(OH)D has been examined, low 25(OH)D levels (less than 15 ng/mL) were associated with increased risk of incident hypertension in both Framingham and the Health Professionals Follow-up Survey, suggesting a potential role for vitamin D in the etiology of hypertension (92, 93).

Hypertension prevalence

Hypertension (defined as blood pressure greater than 140/90 mmHg) is a multi-faceted disease with an estimated prevalence in the United States of 30% (98, 99). However, this disease strikes disproportionately more African Americans over the age of

45 than other demographic groups. In African Americans, over three-quarters of those over age 60 are hypertensive while 40% of men and half of women between the ages of 40-59 are hypertensive (20). Similarly vitamin D deficiency is far more common in African Americans afflicting over 90% of this population (Chapter 4). Data suggest that vitamin D may play a role in reducing atherosclerosis and decreasing blood pressure thus leading to lower cardiovascular mortality (24, 25). However, definitive studies to prove an association between vitamin D and blood pressure and the mechanisms involved have not yet been undertaken. Vitamin D supplementation has been shown to reduce systolic blood pressure in elderly women, yet none have examined vitamin D as a treatment option for aged African Americans with hypertension or pre-hypertension (100).

Prevention of hypertension

Hypertension has severe consequences and requires a lifetime of drug treatment, therefore, interventions to prevent individuals with pre-hypertension from developing hypertension are critical (20, 101). Factors such as tobacco use, obesity, and high dietary salt intake can increase blood pressure (102-104). Since complications arising from increased blood pressure begin at 115/75 mmHg, even a small reduction in blood pressure can reduce the risk of adverse outcomes (105). Lifestyle interventions targeting weight loss, cessation of smoking, and dietary changes are among the first choices when an individual is considered at risk for developing hypertension (101, 106-108). Recent evidence from Nurse's Health Study and Health Professionals Follow-up Study suggest that higher (above 15 ng/mL) vitamin D levels in normotensive individuals may be protective against developing hypertension (92).

Hypertension: treatment and complications

A myriad of treatment options such as diuretics, angiotensin converting enzyme (ACE) inhibitors, beta-blockers, and calcium channel blockers, are available for controlling blood pressure (101). Though many options exist, only 58% of those with a diagnosis of hypertension in NHANES in 1999-2000 were taking some form of blood pressure medication (20). Further, only 53% were adequately controlling blood pressure. Inadequate control of blood pressure can lead to many adverse consequences including myocardial infarction, renal disease, peripheral vascular disease, stroke, impaired vision, chronic pain, and increased mortality (109). Due to cost of medication and regularity with which medication must be taken, many hypertensive individuals are often not compliant with medication regimen (110). Vitamin D deficiency can be easily corrected and often does not require a lifetime of treatment (42).

Hypertension: assessment of blood pressure

An individual is considered hypertensive when he or she has a systolic blood pressure above 130 mmHg for two or more visits to the doctor. A high degree of variability exists when measuring blood pressure (111). Some individuals exhibit increased blood pressure at the clinic (white coat hypertension) which can lead to misclassification of an individual as hypertensive (112, 113). In addition, single measures of blood pressure are highly variable thus leading to increased number of subjects needed to effectively power clinical trials studying blood pressure (112, 114-116). Continuous monitoring of patients using techniques such as 24-hour ambulatory blood pressure (ABP) monitors or home monitors can improve precision and accuracy

when studying blood pressure (114-120). This helps to reduce measurement bias when used in a clinical trial.

Hypertension: systems of blood pressure control

Regulation of blood pressure involves many systems in the body including: cardiovascular, nervous and endocrine systems. Stimulation from the sympathetic nervous system increases the heart rate and constricts the blood vessels thus increasing blood pressure (121). Drugs such as beta blockers counteract increased blood pressure resulting from overstimulation of the sympathetic nervous system (122, 123). The primary blood pressure control system of the endocrine system is the renin-angiotensin aldosterone system (124, 125). This system responds primarily to changes in sodium level in the blood and controls blood pressure by reducing the amount of excreted sodium or by vasoconstriction (126-128).

Several mechanisms exist for controlling blood pressure. Renin is released from the juxtaglomerular cells of the kidney in response to decreased pressure, low sodium levels in the nephron, and increased activity from the sympathetic nervous system (121, 129-131). Renin cleaves angiotensinogen into angiotensin I which is converted to angiotensin II by angiotensin converting enzyme (ACE). Drugs such as ACE inhibitors, angiotensin receptor blockers (ARBs), and renin inhibitors target this system to reduce blood pressure (124, 132, 133).

Lastly the cardiovascular system itself exerts control over blood pressure. Baroreceptors in the aorta and carotid sinus send signals to the SNS which in turn can increase cardiac output and increase blood pressure by releasing chatecholamines

targeting the heart and blood vessels (121, 134). In addition the heart itself participates in regulation of blood pressure through the release of atrial natriuretic peptide (ANP), a peptide involved in salt and water balance (135). The heart also controls arterial pressure by exerting force on the blood as it moves through the heart. Muscles in the heart experiences changes in contractile force mediated by voltage gated calcium channels (135). Calcium channel blockers are used to reduce the contractility of the heart and thus reduce blood pressure (136, 137).

Vitamin D and the renin-angiotensin aldosterone axis (RAS)

Recent clinical and epidemiological studies have demonstrated that higher levels of 25(OH)D are associated with lower blood pressure, though the mechanism through which 25(OH)D lowers blood pressure is not known (100, 138). Results from animal studies suggest reduction in blood pressure is due to the action of $1\alpha,25(OH)_2D$ on the renin-angiotensin system (RAS) (88, 125, 139). $1\alpha,25(OH)_2D$ inhibits renin gene in mice treated with strontium to block $1\alpha,25(OH)_2D$ production (139). These mice develop hypertension that can be reversed upon treatment with captopril, an angiotensin converting enzyme (ACE) inhibitor, suggesting that low $1\alpha,25(OH)_2D$ levels increase blood pressure in mice. Further, wild-type mice in which $1\alpha,25(OH)_2D$ is inhibited have increased renin activity (139). These experiments have also been repeated in mice that lack 1-alpha hydroxylase and again demonstrate the same phenotype that can be rescued upon treatment with $1,25(OH)_2D$ (140). In humans, it is not known if vitamin D repletion resulting in higher circulating levels of vitamin D metabolites is associated with lower renin levels (141-144).

RAS and inflammation

In addition to direct effects on the vascular endothelium and the RAS, $1,25(OH)_2D$ has been shown to reduce inflammatory cytokines such as TNF alpha and IL-6 (76) . Activated T-cells have been shown to increase oxidative stress and subsequently increase angiotensin-II (ang-II) production (145-147). The increase in ang-II production is a direct result of increased oxidative stress. Ang-II is a potent vasodilator and the downstream product of the RAS. Therefore, vitamin D may lower blood pressure is through reduction in inflammation leading to reduction in RAS activation.

$1,25(OH)_2D$ and blood pressure

In an study of individuals with hypertension, $1,25(OH)_2D$ administration resulted in increased blood pressure after 120 minutes of a bolus injection (0.02 ug/kg body weight) (148). However, no change in blood pressure was observed in the non-hypertensive controls. In a Japanese case study blood pressure decreased from 145/96 mm Hg to 128/85 mm Hg as did plasma renin activity after $1,25(OH)_2D$ administration (142). Cross-sectionally, serum levels of $1,25(OH)_2D$ were found to be inversely associated to systolic blood pressure in hypertensives ($r = -0.42$, $P < .02$), (149) and in normotensives (-0.27, $p=0.05$) (150). In rats, it has been shown that higher levels of $1,25(OH)_2D$ lead to increased force generation in response to norepinephrine indicating $1,25(OH)_2D$ may play role in recognition of SNS output (151, 152).

Epidemiologic evidence

Epidemiological evidence suggesting that vitamin D deficiency may exacerbate hypertension has been inconsistent (16, 91, 92, 94, 95, 153-156). One reason for the inconsistency is that each study used different measures (vitamin D intake or circulating 25(OH)D or $1\alpha,25(OH)_2D$) to assess vitamin D as an exposure. It is now known that 25(OH)D is the major marker of vitamin D status and that $1\alpha,25(OH)_2D$ and vitamin D intake are not necessarily representative of 25(OH)D status. This explains why studies using $1\alpha,25(OH)_2D$ as a biomarker have had widely different results when compared with studies using 25(OH)D (16, 153, 156, 157). Similarly, vitamin D intake has not been shown to be associated with hypertension, however, intake in most studies was low and the investigators did not account for sunlight exposure (40, 94, 95). When circulating 25(OH)D has been examined, low 25(OH)D levels (less than 15 ng/mL) were associated with increased risk of incident hypertension, suggesting a potential role for vitamin D in the etiology of hypertension (92, 93).

Clinical trials

A randomized, placebo controlled trial supplementing 145 elderly women with 400 IU cholecalciferol and 600 mg calcium found that systolic blood pressure decreased by 9.3% after eight weeks of supplementation while the placebo group decreased only 4%.7 (100). A second study in the UK conducted in the winter found that supplementing 189 elderly people with one dose of 100,000 IU of cholecalciferol decreased mean heart rate from 74 to 72 beats per minute but did not lower blood pressure (90). However, serum 25(OH)D levels were only raised from 14 to 21 ng/mL which may not be

sufficient to decrease blood pressure. This clinical trial was repeated in Type 2 diabetics. Blood pressure decreased by 14 mmHg when compared with placebo and endothelial function improved (158). A third study testing UVB light vs UVA light (placebo) found that those in the UVB treatment group experienced a 6 mmHg reduction in 24 hour ambulatory systolic and diastolic blood pressure. Further, plasma concentrations of 25(OH)D rose from 20 to 61 ng/mL after six weeks while no change in 25(OH)D was observed in the UVA treated group (138).

Summary

We examined the effect of vitamin D deficiency and strategies for correction of deficiency in two model disease populations particularly sensitive to vitamin D deficiency: hypertension and cystic fibrosis. In chapter 3, the association of 25(OH)D with blood pressure is presented and was published in the American Journal of Clinical Nutrition. Chapter 4 presents the results from the retrospective chart review at the CF center published in Clinical Endocrinology. Chapter 5 provides a description of the clinical trial examining vitamin D repletion strategies in CF patients. Chapter 6 examines the effect of correcting vitamin D deficiency on blood pressure. In Chapter 7 the methods used for each of the previous chapters will be elaborated upon as many of the dissertation chapters were limited due to journal specific requirements. In Chapter 8 we will bring together all of the findings from the previous chapters and make recommendations for future studies.

CHAPTER 3 OPTIMAL VITAMIN D STATUS ATTENUATES THE AGE-ASSOCIATED INCREASE IN SYSTOLIC BLOOD PRESSURE IN WHITE AMERICANS: RESULTS FROM NHANES III

http://www.ajcn.org/cgi/content/full/87/1/136?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&author1=judd&searchid=1&FIRSTINDEX=0&sortspec=relevance&resourcetype=HWCIT

CHAPTER 4 VITAMIN D AND BONE HEALTH IN ADULTS WITH CYSTIC FIBROSIS

http://www3.interscience.wiley.com/cgi-bin/fulltext/120118795/HTMLSTART

CHAPTER 5 TREATMENT OF VITAMIN D INSUFFICIENCY IN CYSTIC FIBROSIS PATIENTS: EVALUATION OF ERGOCALCIFEROL, CHOLECALCIFEROL AND UV LIGHT

Note: this is not the final version. This article has been submitted for publication so will be replaced with a URL when it is published.

Treatment of Vitamin D Insufficiency in Cystic Fibrosis Patients: Evaluation of Ergocalciferol, Cholecalciferol and UV light

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Abstract

Background: The optimal treatment for correcting vitamin D insufficiency in cystic fibrosis (CF) patients has not been established.

Objective: To assess the relative efficacy of three modes of vitamin D therapy: ergocalciferol (D_2), cholecalciferol (D_3), and limited ultraviolet light therapy (UV).

Design: Thirty adult CF subjects with vitamin D insufficiency were randomized into one of 3 treatment arms: D_2, D_3 or UV therapy. Subjects randomized to D_2 or D_3 were instructed to take a 50,000 IU pill each week for 12 weeks and those randomized to UV exposed their lower backs to a portable UV indoor tanning lamp 5 times a week for 12 weeks. Serum was collected for 25(OH)D and parathyroid hormone (PTH) measurements at baseline and at 12 weeks.

Results: Treatment with D_3 and D_2 each raised 25(OH)D levels significantly, from a baseline mean of 21.2 ± 10.18 ng/mL to 47.1 ± 20.5 ng/mL ($p<0.001$) and 24.4 ± 10.3 ng/mL to 32.7± 9.7 ng/mL ($p=0.01$), respectively. Treatment with UV did not raise 25(OH)D levels (23.1 ng/mL to 28.2 ng/mL, p=NS).

Conclusion: This study demonstrates that CF subjects are able to achieve optimal vitamin D status (>30 ng/mL) with either D_2 or D_3 treatment, the latter being the most efficacious. Use of unsupervised UV therapy did not alter vitamin D status. Our results

suggest that vitamin D_3 at a dose of 50,000 units/week for 12 weeks is an effective method to improve vitamin D nutriture in adult CF patients.

Abstract Word Count=239

Introduction

The prevalence of vitamin D insufficiency in patients with cystic fibrosis (CF) has been reported to be as high as 90% (1, 2). This is significantly higher than healthy controls from a similar geographic area (2) or when compared to 25-hydroxyvitamin D [25(OH)D] levels from the Third National Health and Nutrition Examination Survey (NHANES III) (3). This high prevalence occurs despite the routine use of vitamin D supplementation in CF patients (1, 4). Poor absorption of fat soluble vitamins due to pancreatic insufficiency and diminished exposure to sunlight are thought to be among the causes for low vitamin D in this population(5).

Vitamin D insufficiency is associated with decreased bone mineral density (BMD)(6, 7) in patients with CF as well as in other disease states. Low BMD, in turn, is associated with diminished lung function (as measured by forced expiratory volume in the first second or FEV1) in these patients (6). A direct association between vitamin D insufficiency and poor lung function has also been reported in non-CF adults (8, 9). Furthermore, vitamin D is postulated to enhance the innate immune system (10) and improve insulin sensitivity (11), all issues of clinical relevance in the CF population who suffer from CF related diabetes, frequent pulmonary infections and progressive respiratory failure(12).

The best way to overcome vitamin D insufficiency in CF patients has not been established. Current guidelines by the Cystic Fibrosis Foundation Consensus Conference

for vitamin D supplementation with high dose ergocalciferol (vitamin D_2) appear to be inadequate (1). Previous studies in healthy adults (13, 14) have suggested that high dose cholecalciferol is more effective in raising 25(OH)D levels compared to ergocalciferol. In contrast, a recent study showed that at lower daily replacement doses, ergocalciferol and cholecalciferol are equally efficacious in raising 25(OH)D levels in non-CF patients(15). An alternative to oral vitamin D supplementation in CF patients is UV light therapy to produce vitamin D_3 in the skin. UV light therapy circumvents the problem of malabsorption in these patients, and has been demonstrated effective in small pilot studies to improve vitamin D status in subjects with CF (5, 16). A portable UV table lamp can provide the UVB radiation that sunlight can not provide efficiently in northern latitudes during the winter months(6).

The relative efficacy of ergocalciferol, cholecalciferol, and limited UV light therapy in vitamin D replacement of CF patients has not yet been studied. The primary aim of this study was to compare the relative efficacy of these three modes of vitamin D replacement in clinically stable adults with CF.

Materials and Methods

Subjects with CF were recruited from the Emory Cystic Fibrosis Center from November of 2006 to March of 2008. The timing of recruitment was limited to the late fall and winter months in order to minimize the contribution of endogenous vitamin D_3 production from the sun. The study was approved by the Emory Institutional Review board (IRB) and all subjects gave written informed consent. The study was registered at clinicaltrials.gov under study #NCT00450073.

Subjects

Patients were included in the study if they were between the ages of 16 to 70, had a screening serum 25(OH)D level between 10 and 40 ng/mL, and had a confirmed diagnosis of CF by genetic analysis or sweat chloride testing. Exclusion criteria were history of skin disease or cancer, presence of renal or hepatic disease, recent treatment with high dose vitamin D, treatment with prednisone, or a history of more than 6 hospitalizations within the past year. Patients were also excluded if they were planning any trips during the study to sunny climates lasting longer than 2-3 days. Our study included patients with serum 25(OH)D levels up to 40 ng/mL at screening expecting that CF subjects would experience a decrease in 25(OH)D in the winter (17).

Design

Thirty CF subjects were randomized in blocks of 6 to three treatment arms: vitamin D_3, vitamin D_2 or UV light therapy (see Figure 1). The vitamin D_2 50,000 IU and vitamin D_3 50,000 IU capsules were obtained from Pliva, Inc. (East Hanover, NJ) and Tishcon Corp. (Westbury, NY), respectively. A sample of each vitamin D capsule was assayed by an independent laboratory using high-performance liquid chromatography (HPLC) with a reversed phase column. The vitamin D_3 tablets were found to contain 49300 (+/- 900) IU of vitamin D_3 and the vitamin D_2 gel capsules were found to contain 47100 (+/- 1100) IU of vitamin D_2.

For all subjects, serum was collected at baseline (Figure 1). A focused food frequency questionnaire was used to determine mean daily vitamin D consumption by subjects. The questionnaire we used was adapted from a previously published version(18). Subjects randomized to D_2 or D_3 were instructed to take one pill each week for 12 weeks. CF subjects randomized to UV light therapy exposed their lower backs in a seated position to a portable UV indoor tanning lamp (Sperti sunlamp "Del Sol", Crescent Springs, KY) at a distance of 14 inches for 3-10 min depending on their skin type five times a week according to a UV light protocol developed to enhance vitamin D status in CF patients(5). The Sperti sunlamp mimics natural sunlight in that it emits 2-5% UVB between the wavelengths of 290 to 320 nm(5). Subjects were contacted on a weekly basis to ensure compliance with their respective treatment regimens. Subject recall was used to assess compliance. Subjects returned for their final serum collection within 4 weeks of study completion. On their final visit, those subjects randomized to UV light therapy had their backs examined for presence of any burns.

Analytical methods

Serum 25(OH)D_2, 25(OH)D_3, and total 25(OH)D was determined using LC-MS by Quest Diagnostics Incorporated (San Juan Capistrano, CA). PTH was done using ELISA (Immutopics International, LLC, San Clemente, CA). Mean daily intake of vitamin D by subjects was estimated using an 11-item food frequency questionnaire. A serving size for each food item was described to the subjects. Subjects were then asked to recall the number of serving sizes they consumed for each food item within a given day, or week, or month. For each food item, the amount of vitamin D contained in each

serving size was multiplied by the frequency of intake for each subject. Total vitamin D intake was then calculated, and averaged to represent mean intake per day.

Statistical methods

Data was first examined to ensure normality. All recorded variables were normally distributed. We used SAS 9.2 (SAS Institute Cary, NC) to perform all analyses. The study was originally powered to detect a difference of 7.5 ng/mL in 25(OH)D between groups. We used Fisher's Exact Test (PROC FREQ) to calculate p-value comparing count data because many of our cells had less than 5 individuals. Simple ANOVA was used to compare differences in mean covariates by treatment groups and ANACOVA to compare mean differences when controlling for covariates (PROC GLM and PROC MIXED). Least squares means were calculated for all means reported using a Bonferroni correction whenever repeated measures were present. To determine whether initial 25(OH)D status effected change in 25(OH)D we used Pearson's correlation (PROC CORR). When considering our final models, we used initial 25(OH)D status, age, type of CF mutation, serum calcium, vitamin D intake, use of vitamin supplement as potential covariates. We tested for interaction with CF mutation, and use of vitamins however significant interaction was not present. We conducted analyses using intent to treat.

Results

Subject demographics

We screened 33 individuals. Of those, 30 entered the study, and one patient each was lost to follow-up in the UV and D_3 groups. The mean age, BMI, FEV_1 percent predicted and use of pancreatic enzyme replacement was not significantly different among treatment groups (Table 1).

Baseline Vitamin D status

Sixty eight percent of our study population were vitamin D insufficient (25(OH)D < 30ng/ml) at baseline All groups had similar dietary intakes of vitamin D, between 369 IU and 587 IU (Table 1). Greater than 80% of the subjects reported daily use of a multivitamin which did not differ among the three groups. The initial 25(OH)D ranged between 21 and 24 ng/mL and was not different among the three treatment groups (p=0.8). Twenty-two percent of the subjects in the D_3 and UV groups were vitamin D sufficient (25(OH)D >30 ng/mL) while 40% of subjects in the D_2 group were sufficient (Table 2).

Factors associated with increases in 25(OH)D

The initial 25(OH)D level was associated with the rate of change in 25(OH)D (Table 3). The negative correlation coefficient indicates that those who start out with the highest serum 25(OH)D levels exhibited the smallest increase in 25(OH)D throughout the study period in response to oral vitamin D. We therefore controlled for initial 25(OH)D level when examining the mean rates of change (Figure 2). When controlling for initial

serum 25(OH)D status, those receiving D_3 had the largest increase in serum 25(OH)D when compared with the other two treatments ($p<0.01$) (Table 4). Those receiving D_3 also had the largest increase in serum 25(OH)D_3 ($p<0.001$) (Table 4). Serum 25(OH)D_2 concentrations also increased in those receiving D_2 ($p=0.02$) and decreased but not significantly in those receiving D_3 or UV. We examined age, BMI, sex, CF mutation, skin type, and vitamin D intake as potential covariates though none were significant at the 5% level.

Total Serum 25-hydroxyvitamin D concentrations after D_2, D_3 or UV light

Subjects randomized to oral forms of vitamin D (D_2 or D_3) exhibited significant increases in total serum 25(OH)D concentrations (Table 4 and Figure 2). The most robust increase in total serum 25(OH)D occurred in the D_3 treated group, where the final total serum 25(OH)D was 47.1 ± 3.1 ng/mL, resulting in a significant total increase of 25(OH)D of 25.3 ± 2.8 ng/mL ($p<0.001$). In the D_2 treated group, the total increase in 25(OH)D concentration was less impressive at 8.8 ± 2.8 ng/mL ($p=0.04$) (Figure 3A). In the D_3 treated group, 100% of the subjects reached vitamin D sufficiency (25(OH)D > 30 ng/mL) as compared to only 60% of those treated with D_2 and 22% of those treated with UV ($p<0.001$). In those randomized to receive UV treatment, the final total serum 25(OH)D was 28.2 ± 3.2ng/mL for a non-significant change of 5.2 ± 3.4ng/mL ($p=0.25$) (Figure 3A).

25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 concentrations after D_2, D_3 and UV light

As expected, subjects who received D_3 had significant increases in serum 25(OH)D_3 concentrations from 16.9 to 46.0 ng/mL ($p<0.001$). In the D_2 treated group the serum 25(OH)D_2 level increased by 17.7 ± 3.3 ng/mL but the 25(OH)D_3 level decreased by 8.9 ± 3.3 ng/mL. This decrease in serum 25(OH)D_3 concentrations was significant compared to D_3 ($p<0.001$) (Figure 3B). Those subjects who received UV light did not have a significant increase in 25(OH)D_3 ($p=0.09$).

The 25(OH)D_2 concentrations in subjects receiving D_3 and UV started low initially (over half of the sample in both groups had levels that were not detectable at the beginning of the intervention) at 4.3 and 1.9 ng/mL and did not significantly decline with therapy ($p = 0.12$ and 0.17 respectively).

Parathyroid hormone concentrations after D_2, D_3 and UV light

Initial PTH levels in D_3 treated subjects were nearly half that of D_2 and UV treated subjects ($p=0.01$) (Table 2). There was no signifcant difference in the change in PTH between treatment groups D_2 and D_3 ($p=0.86$). However, when we controlled for initial PTH level, the change in PTH between D_2 and UV was siginficant ($p=0.05$) while the change between D_3 and UV groups was not different ($p=0.08$) (Figure 4).

Safety

There were no reported adverse events nor were there any cases of hypercalcemia. All of the individuals randomized to a pill (D_3 or D_2) were 80% compliant or better. Only 55% of those on UV therapy were compliant with therapy more than 80% of the time. When we only considered 5 out of 9 subjects who were adherent with UV therapy then the rise in 25(OH)D was similar to those treated with D_2 ($p=0.93$).

Discussion

In this prospective randomized study of three different regimens to correct vitamin D insufficiency, we found that both oral D_2 and D_3 was effective in raising 25(OH)D concentrations with D_3 demonstrating the greater response. UV therapy was limited by poor adherence to the study protocol but was equally as effective as oral D_2 after exclusion of non-adherent subjects. As expected, PTH levels followed the same trend, with the greatest decrease in PTH level occurring in the vitamin D_3 treated group, followed by D_2 and then UV light therapy.

Our results are in agreement with previous studies in healthy subjects demonstrating superiority of vitamin D_3 over vitamin D_2 in raising 25(OH)D levels (13, 14). Trang et al (14) showed that when subjects were given 4000 IU vitamin D_3 for 14 days they were able to raise their 25(OH)D levels 1.7 times more than subjects given the same dose and duration of vitamin D_2 (increase of 9.3 ng/mL vs. 5.5 ng/mL). Armas et al (13) compared vitamin D_2 to vitamin D_3 when given as a single dose of 50,000 IU. Despite a similar rise in 25(OH)D levels over the first 3 days for both vitamin D_2 and vitamin D_3, suggesting equal absorption, 25(OH)D levels in the vitamin D_2 group rapidly fell back to baseline by the second week. The area under the curve (AUC) by day 28 was 204.7 ng.d/mL for vitamin D_3 as compared to 60.2 ng.d/mL for vitamin D_2.

The Cystic Fibrosis Foundation consensus statement from 2005 (19) recommends daily oral supplementation with ergocalciferol at a daily dose of 400 to 800 IU. Several

studies in CF patients, however, have shown such doses to be inadequate at optimizing 25(OH)D levels. A prospective study of 20 CF patients showed that after one year of treatment with 800 IU vitamin D_3 daily, none of the patients were able to raise their 25(OH)D levels above 30 ng/mL (70 nmol/L)(20). Rovner et al (2) showed that 90% of CF patients remained vitamin D insufficient despite routine supplementation with 800 IU a day. Furthermore, in a recent large retrospective study in CF patients (4), it was demonstrated that despite daily cholecalciferol supplementation (mean dose 647 ± 339 IU) which increased 25(OH)D levels significantly (mean of 14.2 ng/mL to 25ng/mL), only 18% of CF patients were able to achieve 25(OH)D levels above 30 ng/mL.

For vitamin D insufficient CF patients (25(OH)D levels <30ng/ml), the consensus statement recommends treatment with ergocalciferol 50,000 IU either once or twice weekly. A study by Lark et al demonstrated that when ergocalciferol was given to CF subjects as a single dose of 100,000 IU, they were only able to absorb half as much of the ergocalciferol when compared to controls. Also, CF subjects were less efficient at converting ergocalciferol to 25(OH)D compared to controls(21). Boyle et al (1) demonstrated that CF patients with vitamin D insufficiency only 8% of subjects were able to reach 25(OH)D levels >30 ng/mL after 8 weeks of treatment with weekly 50,000 IU of ergocalciferol. Those that did not achieve vitamin D sufficiency were treated for another 8 weeks, yet none was able to achieve vitamin D sufficiency. Green et al demonstrated that treatment with ergocalciferol once, twice or three times a week for 8 weeks was able to raise 25(OH)D levels above 30 ng/mL in only 33%, 26%, and 43% respectively (22). Furthermore, in this study, the proportion of patients with follow-up 25(OH)D levels >30 ng/mL were the same irrespective of whether or not they had been

treated with ergocalciferol. Based on these data, the recommendations for maintenance of vitamin D status and correction of vitamin D insufficiency should be revised to higher levels, preferably with D_3.

In our study, CF subjects taking vitamin D_2 are able to raise their total 25(OH)D concentrations levels, however this is attenuated by the corresponding decrease in $25(OH)D_3$. Circulating vitamin D exists mostly as $25(OH)D_3$ representing vitamin D from cutaneous and most dietary sources of vitamin D. Less circulating vitamin D exists as $25(OH)D_2$ which represents vitamin D from dietary sources fortified with ergocalciferol only, which is limited to very few foods. This finding of lowered $25(OH)D_3$ after vitamin D_2 therapy is of concern since ergocalciferol is most commonly available by prescription to correct vitamin D insufficiency in the United States and is recommended by the CF foundation for correction of vitamin D insufficiency. Based on the studies of Trang et al. (14) and Armas et al. (13), some have suggested that ergocalciferol not be used as a supplement to correct vitamin D insufficiency (23).
A recent study by Holick demonstrated equal efficacy of vitamin D_2 and vitamin D_3 in raising 25(OH)D levels (15). However, vitamin D_2 and D_3 were given daily at much lower doses (1000 IU a day) in that trial and to subjects without chronic malabsorption, as in the CF patients we studied. This suggests that there may be differences in vitamin D_2 and D_3 when given in pharmacologic (>10,000 IU) doses rather than in more physiologic doses (<2,000 IU), although such studies in CF have not been performed. A recent study (24), demonstrated that at vitamin D_3 intake doses <2000 IU per day there is rapid conversion of vitamin D_3 to $25(OH)D_3$, but at doses >2000 IU per day vitamin D_3 concentrations can be detected in the serum along with a rise in $25(OH)D_3$. It has been

demostrated that vitamin D_3 binds vitamin D binding protein (DBP) more avidly than vitamin D_2(25) thus allowing vitamin D_3 to have a longer circulating half-life. One could infer from these two studies that at lower intake levels, vitamin D's metabolism becomes independent of how avidly it binds DBP. At higher intake doses, the differential avidity for DBP of vitamin D_3 and vitamin D_2 becomes more evident. Finally, vitamin D_2 may activate the pregnane X receptor (PXR) which in turn upregulates the CYP24, the enzyme that catabolizes vitamin D. The PXR is a recently described nuclear receptor that is important for the detoxification of foreign substances in the body (26). This could be plausible given that vitamin D3 is the endogenously produced vitamin D compared to vitamin D_2 which is obtained from irradiated fungi or yeasts.

We found that UV light therapy did not significantly raise 25(OH)D levels. One explanation for the lack of a significant rise in 25(OH)D levels with UV light therapy could be attributed to the high non-adherance rate of subjects assigned to this treatment modality. Over half of the eligible subjects randomized to receive UV light therapy were not adherent with therapy per self-report. When we excluded those individuals, the mean 25(OH)D increase in the UV treated group was 11 ng/mL ($p=0.04$); however, only one of the individuals reached vitamin D sufficiency. In addition, our subjects exposed only their backs to the sunlamp, a much smaller surface area, when compared to a previous study in CF patients showing a change in 25(OH)D from 21 to 44ng/ml after 8 weeks of whole body tanning(16).

Our study has limitations. We studied adult CF patients with clinically stable disease and UV light therapy was not supervised . Thus, adherence to UV light therapy was by subject report only. Also, our study was a short term study and does not evaluate long

term effects of vitamin D treatment on bone mineral density and efficiency of intestinal calcium absorption. It is not known what level of 25(OH)D is required to prevent bone loss and maximize calcium absorption in CF. Optimal levels of 25(OH)D have been extrapolated from non-CF populations. A recent study in pediatric CF patients suggests that these patients have normal baseline intestinal calcium absorption that did not improve further with daily 2000 IU D_3 treatment.
(27).

We conclude that oral vitamin D_3 given 50,000 IU once a week for 12 weeks is the most efficacious method to increase 25(OH)D and lower PTH values in CF subjects who are vitamin D insufficient. Ergocalciferol and UV light were not as effective in our study and should be reserved as alternative therapies. The optimal means to correct low vitamin D in patients with CF is not yet determined. However, this study along with others suggests that D_3 should be considered over D_2. Since vitamin D status is often low in CF patients, a more preventative approach to treatment of vitamin D status should be adopted especially during the winter.

Acknowledgements

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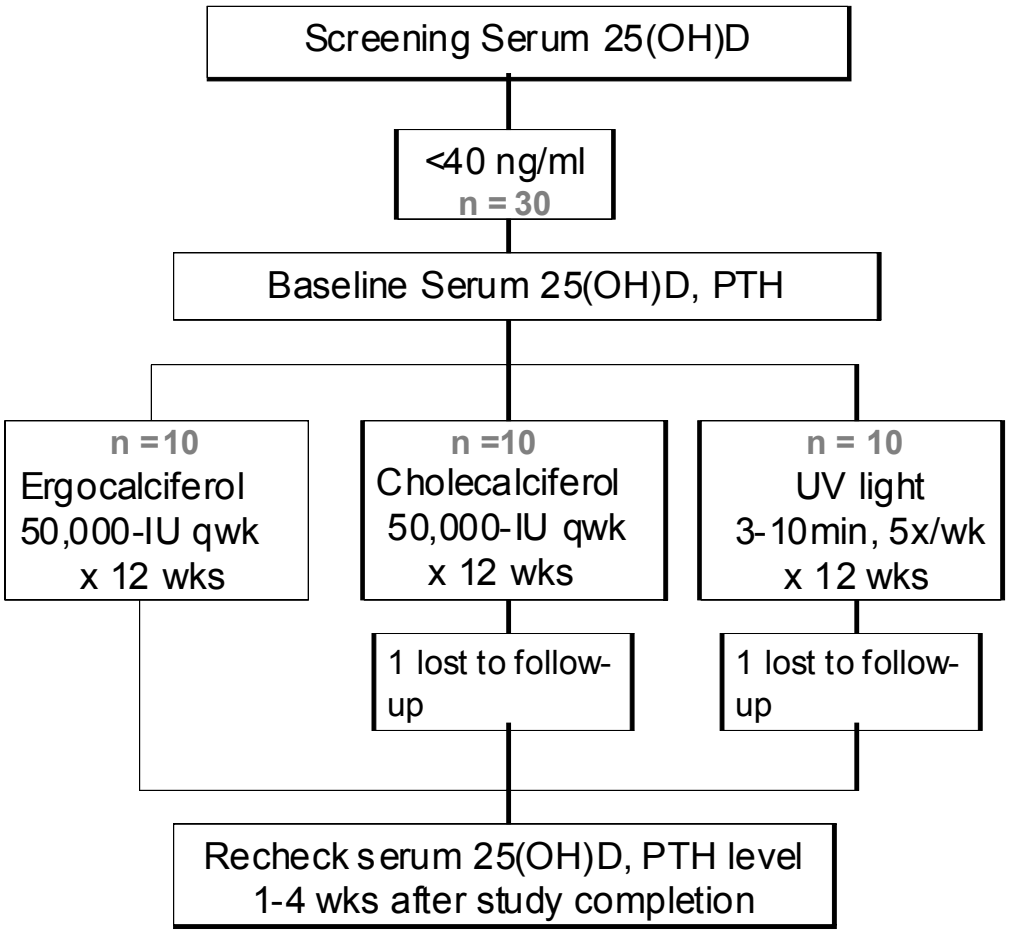

Figure 1 Subject recruitment and randomization

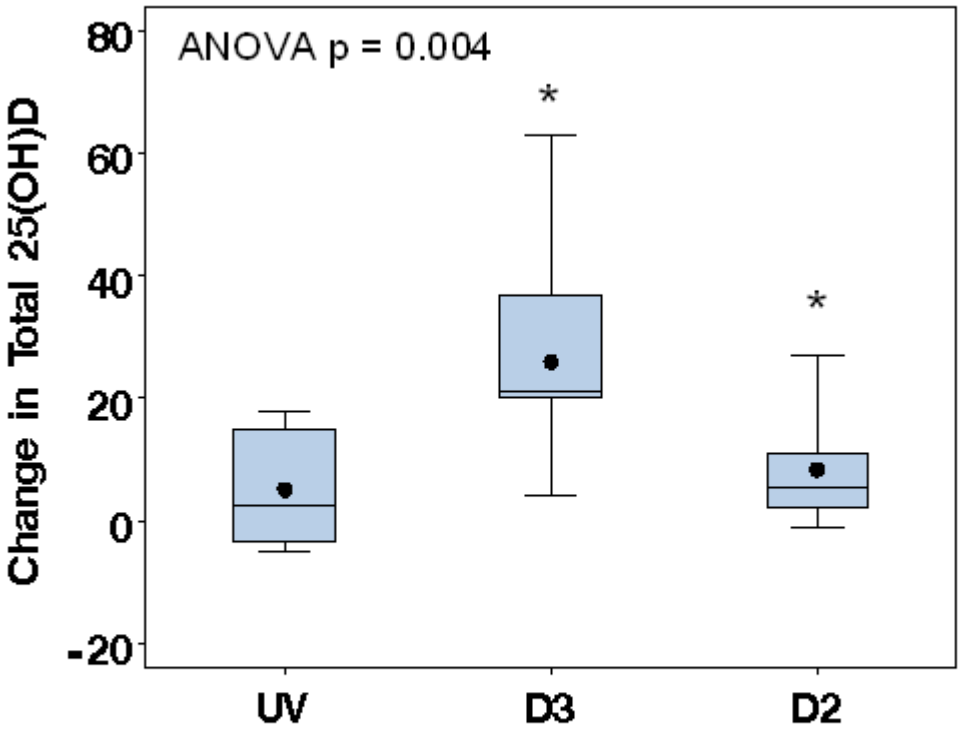
ANOVA p = 0.004
Change in Total 25(OH)D
80
60
40
20
0
-20
*
*
UV
D3
D2

Figure 2 Boxplot of mean change in total 25(OH)D (ng/mL) by treatment group

Subjects were randomized to receive ergocalciferol 50,000 IU once a week for 12 weeks (D_2), cholecalciferol 50,000 IU once a week for 12 weeks (D3), or UV light with a Sperti lamp five times a week for 12 weeks (UV). Data presented are raw data and are not controlled for initial 25(OH)D levels. Error bars represent maximum and minimum value, dot represents the mean and line represents median 25(OH)D levels. Blue box represents interquartile range (25^{th} percentile to 75^{th} percentile) Those treated with D3 had a significant increase in total 25(OH)D level ($p=0.007$ when compared with UV and $p=0.01$ when compared with D_2). Those treated with UV and D_2 experienced similar increases in 25(OH)D ($p=0.85$). * $p<0.05$ using PROC GLM with Bonferroni correction

Figure 3A

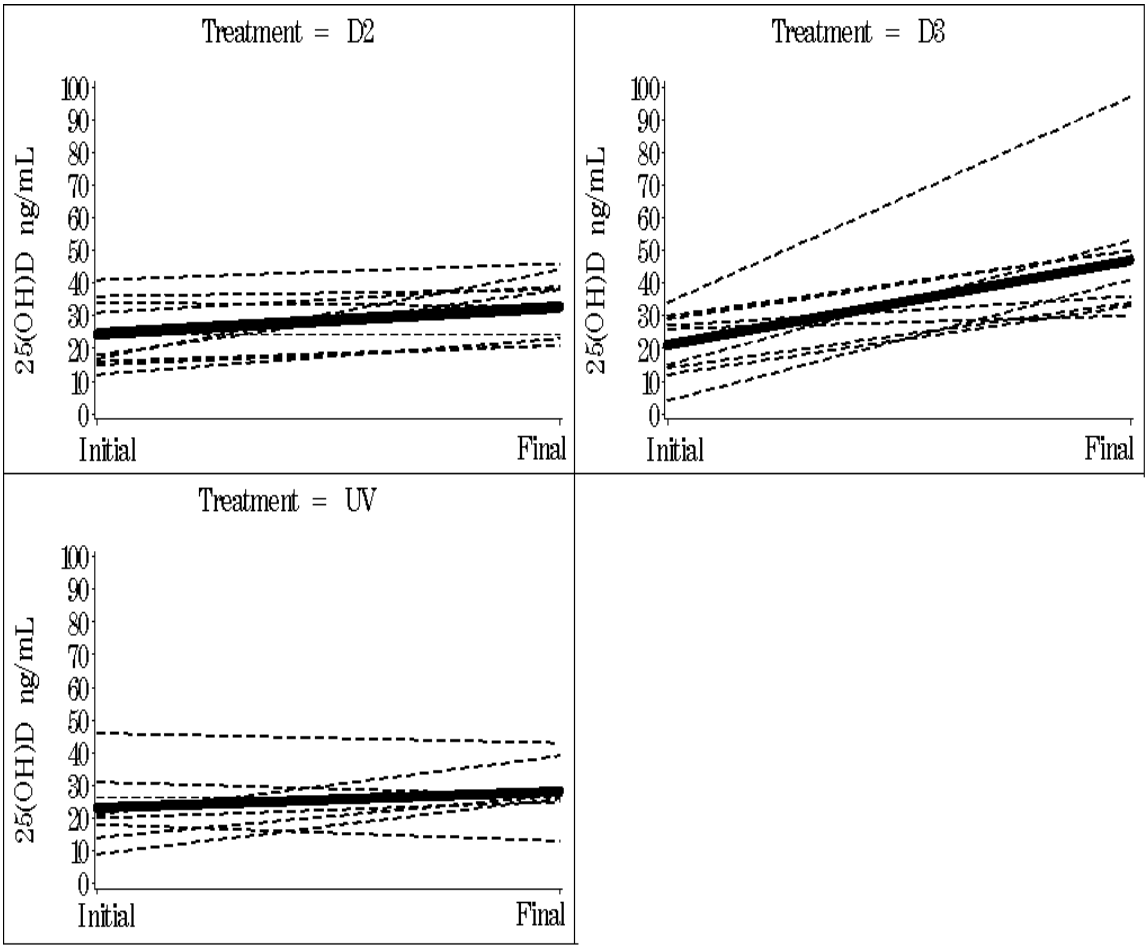
Treatment = D2
Treatment = D3
Treatment = UV
25(OH)D ng/mL
100
90
80
70
60
50
40
30
20
10
0
Initial
Final

Figure 3B

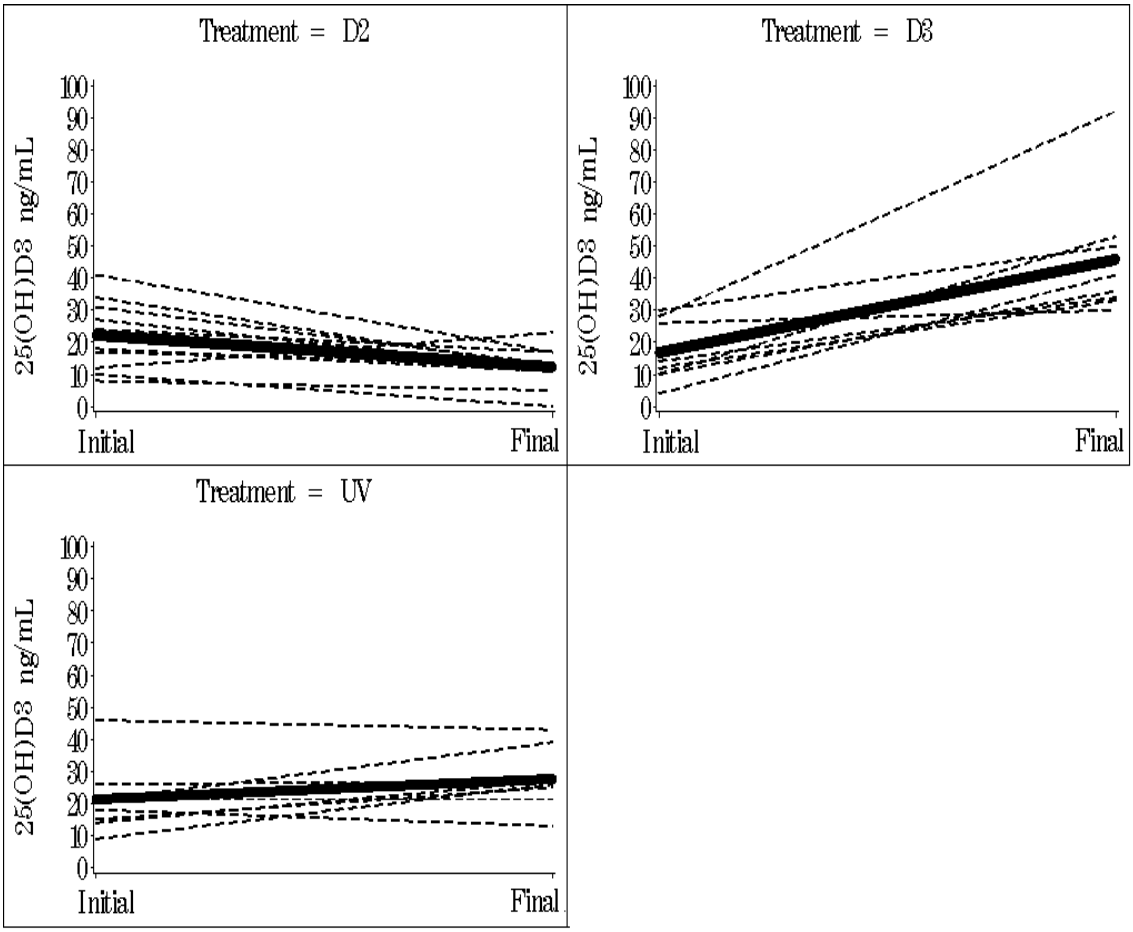
Treatment = D2
Treatment = D3
Treatment = UV
25(OH)D3 ng/mL
100
90
80
70
60
50
40
30
20
10
0
Initial
Final

Figure 3C

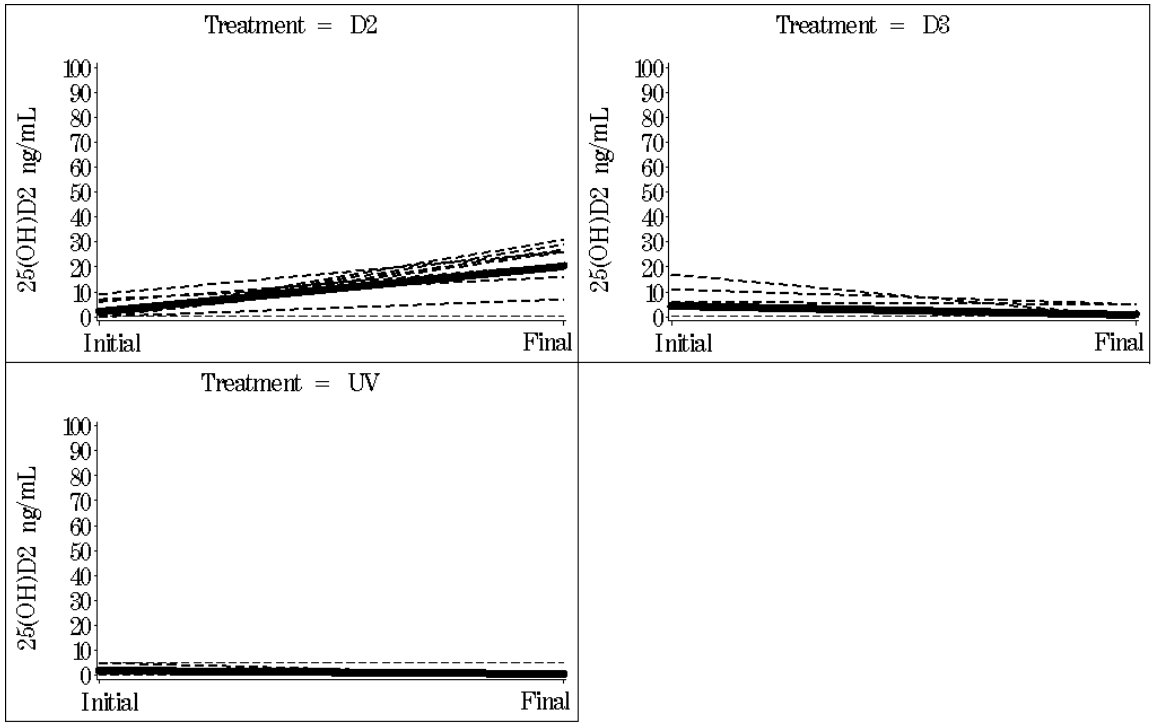
Treatment = D2
Treatment = D3
Treatment = UV
25(OH)D2 ng/mL
100
90
80
70
60
50
40
30
20
10
0
Initial
Final

Figure 3 Change in total 25(OH)D, 25(OH)D_3 and 25(OH)D_2 by treatment group. Bolded line indicates predicted value for the group using PROC MIXED for mixed linear models and controls for initial levels. Dashed lines represent individual change. The total 25(OH)D concentrations were significantly higher in those subjects receiving D_3 and D_2 but not in subjects receiving UV (Figure 3A). The 25(OH) D_3 concentrations were significantly higher in subjects receiving D_3 and significantly lower in subjects receiving D_2. There were no significant changes in 25(OH) D_3 concentrations in those receiving UV (Figure 3B). The 25(OH)D_2 concentrations were significantly higher in subjects receiving D_2 and not in subjects receiving D_3 or UV (Figure 3C).

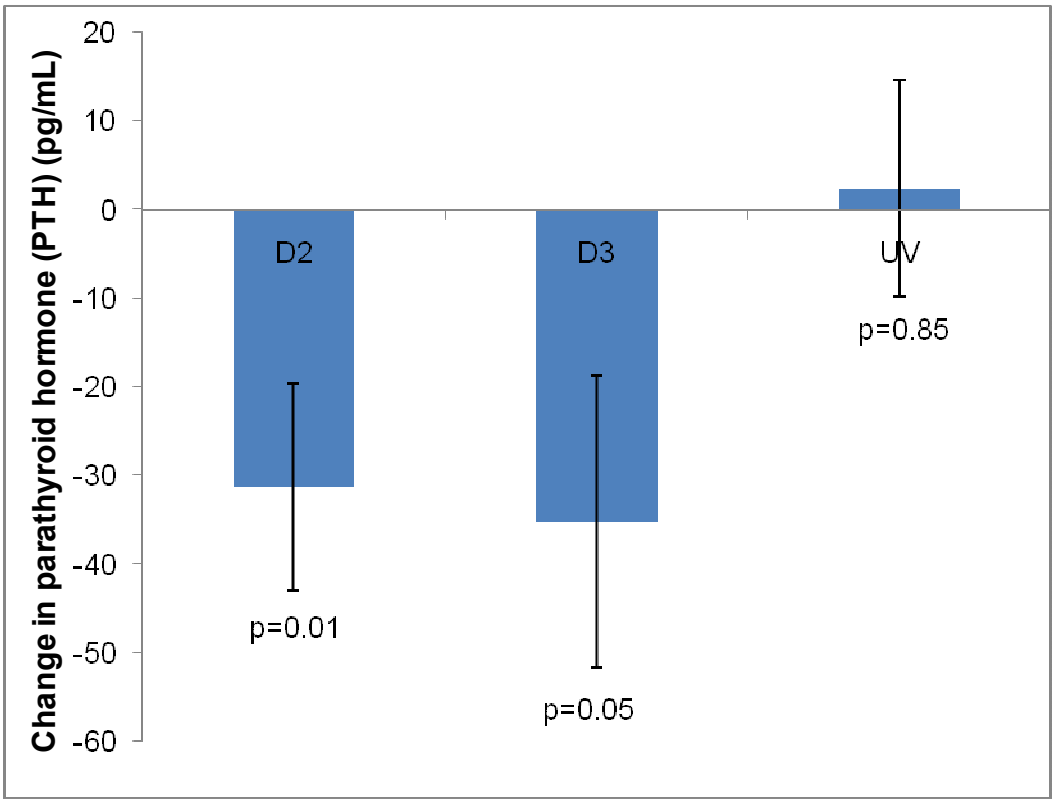

Figure 4 Change in serum parathyroid hormone (PTH) concentrations in response to treatment with ergocalciferol 50,0000 IU once a week for 12 weeks (D2), cholecalciferol 50,000 IU once a week for 12 weeks (D3) or UV light with a Sperti lamp 5 times a week for 12 weeks (UV). Change in PTH were controlled for initial PTH level. Mean change was calculated using least squared means and a Bonferroni correction. Subjects receiving both D_2 and D_3 had significantly reduced PTH levels. There was no signifcant difference in the change in PTH between treatment groups D_2 and D_3 (p=0.86) however the change between D_2 and UV was significant (p=0.05) while the change between D_3 and UV did not reach significance (p=0.08).

Table 1 Baseline characteristics of adult cystic fibrosis patients

	UV	D_3	D_2	p value
Total	9	9	10	
Age (years) ±Std Dev	33.3±15.7	35.7±12.5	27.9±11.9	0.43‡
Sex n (%)				
Male	4	4	5	
Female	5	5	5	0.52*
BMI (kg/m^2)	23.7±3.9	22.5±4.2	22.7±2.9	0.76‡
Pre-Treatment predicted %FEV1 n (%	72.4±28.4	65.5±24.5	76.5±25.8	0.66‡
Reported MVI/ADEK/Ca+D use n (%)	8 (88.9%)	8(88.9%)	8(80%)	0.68*
Average Daily Vitamin D intake (IU)	370 ± 218	456 ± 290	587 ± 321	0.26‡
Mutations				
Homo 508	1	2	4	
Hetero 508	6	3	3	

Neither	2	3	3	0.56*
Initial Serum Calcium	9.0±0.45	9.1±0.28	9.4±0.42	0.05‡
Reported Pancreatic Enzyme Use n (%)	9 (100%)	8 (88.9%)	10 (100%)	0.24*

‡p – ANOVA

* p – Fisher's Exact Test

Values are reported as means plus or minus standard deviations

Table 2 Vitamin D, Calcium and PTH levels before and after intervention

	UV	D_3	D_2	p*
Initial 25(OH)D	22.6 ± 10.8	21.2 ± 10.18	24.4 ± 10.3	0.8
Final 25(OH)D	28.3 ± 9.2	47.1 ± 20.5	32.7 ± 9.7	0.03
% Sufficient				
Initial	22%	22%	40%	0.09
Final	22%	100%	60%	0.003
Initial Ca	9.01 ± 0.45	9.06 ± 0.28	9.44 ± 0.42	0.04
Final Ca	8.97 ± 0.38	8.87 ± 0.50	9.40 ± 0.43	0.24
Initial PTH	111.1 ± 45.0	49.8 ± 46.7	129.9 ± 47.3	0.01
Final PTH	110.0 ± 37.6	40.0 ± 47.6	86.1 ± 40.8	0.02

Values are reported as means plus or minus standard deviations

*p – ANOVA

Table 3 Pearson's correlation between initial 25(OH)D levels and changes in 25(OH)D levels

	Δ 25(OH)D_2	**Δ 25(OH)D_3**	**Δ 25(OH)D**
Initial 25(OH)D_2	**-0.38**	0.24	0.02
Initial 25(OH)D_3	0.34	**-0.42**	-0.29
Initial 25(OH)D	0.18	-0.32	-0.28

bolded indicates significance with Pearson's correlation

Table 4 Mean change in 25(OH)D using mixed linear model to account for initial levels

	UV	D_3	D_2
Initial 25(OH)D	23.1	21.2	24.4
Final 25(OH)D	28.2	47.1	32.7
p*	0.25	<0.001	0.04
Initial 25(OH)D_2	1.9	4.3	2.2
Final 25(OH)D_2	0.6	1.1	20.4
p*	0.39	0.38	<0.001
Initial 25(OH)D_3	20.2	16.9	22.2
Final 25(OH)D_3	27.1	46.0	12.3
p*	0.11	<0.001	0.03

Values are reported as means and were calculated using PROC GLM with a repeated statement

*p compares initial and final levels using least squares means regression

CHAPTER 6 HYPERTENSION CLINICAL TRIAL

Evaluation of vitamin D_3 and $1,25(OH)_2D$ to lower blood pressure in hypertensive subjects: a pilot study

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Key Terms: vitamin D, blood pressure treatment, hypertension, calcitriol, cholecalciferol, renin

Running Title: Treatment of blood pressure with vitamin D

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Abstract

We investigated the effect of vitamin D repletion on resolution of hypertension in a pilot study. Subjects were recruited from the Atlanta Veterans Affairs Medical Center in Decatur, GA. Subjects received a total dose of 600,000 IU D_3 or placebo (n=3) taken weekly either over 3 weeks (n=2) or 12 weeks (n=3) or were given 0.5 ug calcitriol (n=2) taken twice daily for one week. We used a 24 hour ambulatory blood pressure monitor to assess blood pressure. The subjects receiving calcitriol experienced a 13% reduction in average systolic blood pressure while subjects on placebo group only decreased by 4% ($p<0.001$). One week after conclusion of calcitriol therapy blood pressure returned to pretreatment levels in this group. In comparison, mean systolic blood pressure decreased by 5% (136 ± 16.9 initial and 129 ± 15.9 follow-up, $p<0.001$) in the group using D_3 over 12 weeks. Compared with placebo, only the decrease in 24 hour blood systolic? pressure was significant ($p<0.01$). There was not a reduction in blood pressure in the group using D3 over three weeks. Sub-analysis demonstrated that subjects experiencing the largest reduction in blood pressure under treatment with vitamin D_3 had the highest plasma renin activity. Thus, calcitriol had a strong rapid effect on blood pressure reduction while the effect of vitamin D_3 may be limited to individuals with high plasma renin activity. Vitamin D therapy may be an effective intervention for reducing blood pressure and needs to be explored further in larger controlled studies.

Introduction

Inadequate blood pressure control predisposes an individual for increased risk of stroke, kidney disease, and heart disease (1, 2). Two-thirds of Americans are not controlling blood pressure to within normal levels despite knowledge they had hypertension (3). Further, blacks are more likely than whites to have uncontrolled hypertension even though they were more likely to be aware of status as a hypertensive (4). Therefore, adjunctive intervention strategies may be necessary to help improve hypertension control.

Cross-sectional studies have demonstrated that higher levels of 25-hydroxyvitamin D (25(OH)D), are associated with lower blood pressure (5, 6). Low 25(OH)D levels (less than 42.5 nmol/L) were associated with increased risk of incident hypertension in both Framingham and the Health Professionals Follow-up Survey, suggesting a potential role for vitamin D in the etiology of hypertension (7, 8). Further, clinical studies suggest vitamin D lowers blood pressure (9). Together these studies indicate that vitamin D may be both protective and therapeutic in blood pressure management.

Vitamin D acts as a negative regulator of renin in mice studies (10). Vitamin D receptor knock-out mice develop hypertension that is reversed with captopril, an angiotensin converting enzyme (ACE) inhibitor. Other models of vitamin D deficient mice (1-alpha hydroxylase knockout or $1,25(OH)_2D$ depleted mice) also develop

hypertension which is reversed with treatment with $1,25(OH)_2D$ (11, 12). In all three models, the mice go on to develop high renin hypertension, suggesting increased blood pressure is through activation of the RAS.

We therefore sought to determine whether vitamin D supplementation would lower blood pressure in a racially mixed population. We randomized subjects to receive calcitriol, vitamin D3 or placebo. We examined average systolic and diastolic blood pressure and heart rate using a 24 hour ambulatory blood pressure monitor. We further examined urinary calcium excretion and plasma renin activity as biomarker endpoints.

Methods: Vitamin D_3 treatment in hypertensive subjects: a pilot dose ranging study

We tested a regimen of vitamin D_3 in hypertensive subjects to determine its safety and efficacy. We randomized ten subjects to vitamin D and five to placebo. This study was approved by the Emory University Institutional Review Board and Veteran's Affairs Research and Development Committee. The study is registered at clinicaltrials.gov under study number NCT00459563.

Blood collection and quality assurance

Blood was collected at the screening visit, baseline, and final visits. Blood was collected and immediately centrifuged into serum or plasma which was aliquoted into cryovials and stored in a -70C freezer until further analysis. Serum 25(OH)D was determined by ELISA (IDS Ltd, Fountain Hills, AZ) and plasma renin activity (PRA) was determined by RIA (Quest Diagnostics, San Jose, CA). Baseline and follow-up samples from each patient were tested in the same batch along with known standards to ensure quality of test. Urinary creatinine, potassium, calcium and sodium levels were measured by standard hospital methods.

Blood pressure assessment

Blood pressure was measured at screening with the subject sitting quietly in the examination room for 5 minutes. The purpose of this measure was just to determine eligibility for the study. For baseline and follow-up visits, subjects were requested to

wear an ambulatory blood pressure (ABP) (Spacelabs 90207) for 24 hours. The first reading from the ABP monitor was compared to the seated blood pressure measure to ensure ABP was functioning properly (within 10 mmHg). When the subject returned 24 hours later the ABP was rechecked against the seated measure. Subject blood pressure measures were downloaded as soon as the monitor was returned and monitor was initialized for each subject within 24 hours of subject appointment. The blood pressure cuff inflated every 15 minutes during the day and every 30 minutes at night. If the subject did not have at least 80% successful readings, the results were excluded from analysis.

Methods: Evaluation of vitamin D_3, 1,25$(OH)_2$D or placebo: a pilot randomized controlled trial

We studied two forms of vitamin D (vitamin D_3 and 1,25$(OH)_2$D) on blood pressure. The single blinded, randomized, controlled, clinical trial was conducted between April 2008 and August 2008.

Participants

A total of 31 subjects were screened, 15 were consented, and 9 were enrolled. Six subjects (2 in vitamin D_3, 2 in 1,25$(OH)_2$D and 2 in placebo) completed all study requirements over the three month period. This study was approved by the Emory University Institutional Review Board and Veteran's Affairs Research and Development Committee. The study is registered at clinicaltrials.gov under study number NCT00459563.

In order to be eligible for the study, subjects had to be over the age of 30, self-define as black or African American, have vitamin D levels between 25 and 75 nmol/L, and have systolic blood pressure between 130 and 150 mmHg. Subjects were excluded for hypercalciuria, hyperparathyroidism, current use of more than three anti-hypertensive medications, inability to understand the consent form, inability to return blood pressure monitor within 24-48 hours after visit, history of alcohol abuse, current alcohol consumption of greater than two drinks per day, diagnosis of chronic kidney disease,

diagnosis of HIV, history of heart disease, history of stroke, inability to comply with study protocol, current treatment for cancer, narcotic dependence, current use of vitamin D analog, and current use of greater than 2000 IU of vitamin D. In addition subjects were excluded if they did not show up for more than three screening visit and/or baseline visit.

Study medication and compliance

Subjects received $1,25(OH)_2D$ manufactured by Pliva Inc. (East Hanover, NJ), vitamin D_3 or placebo manufactured by Tishcon (Westbury, NY). To ensure compliance, we directly observed subjects take vitamin D_3 200,000 IU once weekly for 3 weeks. Vitamin D_3 and placebo were taken orally and were identical in shape and color. Given the short half life of $1,25(OH)_2D$ (~8 hours), subjects took $1,25(OH)_2D$ twice daily for one week. Subjects consumed the first and last pill in the presence of study personnel but consumed the remaining pills at home. Subjects brought bottles containing $1,25(OH)_2D$ with them to the final visit to assess compliance with medication.

Study personnel were blinded to D_3 and placebo as were subjects; however, due to differences in dosing regimen study personnel were not blinded if subject was randomized to $1,25(OH)_2D$. Subjects were blinded to study drug but were told at the conclusion of the study which drug they were receiving. We used similar methods to assess blood pressure, collect and store blood and test biomarkers as those described in the pilot study above.

Statistical Analysis

Data was analyzed using mixed linear models to account for correlation of repeated measures within the same subject. We used Microsoft Excel and SAS 9.1 (SAS Institute, Cary, NC) for all data analyses. Data from the ABP monitors was downloaded using software provided by the manufacturer and was then transferred to Excel using an encrypted spreadsheet. Blood pressure averages were obtained using Microsoft Excel and then transferred into a SAS data set. Data was analyzed using PROC MIXED with a REPEATED statement to account for repeated measures within the same subject. We also used PROC GLM to calculate least squared means controlling for initial blood pressure and used a Bonferroni correction. Average waking blood pressure was used as the main outcome variable. We also examined changes in biomarkers between treatment and control groups and examined ratio of daytime to nighttime blood pressure changes. All analysis was done using intent to treat.

Results

Pilot dose ranging study

Although we randomized ten subjects at the start of the study, four subjects did not begin treatment because of failure to attend the baseline visit. Therefore, we had baseline measures for six total subjects: four were given vitamin D_3 and two were given placebo. One of the subjects receiving placebo refused to come back in for follow-up and one subject receiving vitamin D_3 refused to give a blood sample on follow-up, so we were left with four subjects having both ABP measures and serum. Serum 25(OH)D increased significantly in vitamin D_3 group compared with placebo (71 nmol/L vs 17 nmol/L, $p<0.001$) (Table 6.1).

Blood pressure changes

In the vitamin D_3 group (n=3), the average 24 hour blood pressure decreased by 5% (136 ± 16.9 initial and 129 ± 15.9 follow-up, $p<0.001$) while the daytime blood pressure decreased by 7%(139 ± 15.8 initial and 129 ± 14.6 follow-up, $p<0.001$). In comparison the subjects receiving placebo experienced a 2% (128 ± 11.4 initial and 125 ± 8.7 follow-up, $p=0.20$) drop in 24 hour blood pressure and a 4% (130 ± 11.7 initial and 125 ± 8.7 follow-up, $p<0.01$) drop in daytime blood pressure. Compared with placebo only the drop in 24 hour (not daytime) blood pressure was significant ($p<0.01$). From

these data we concluded our regimen of vitamin D was adequate for restoring vitamin D status.

Evaluation of vitamin D_3, $1,25(OH)_2D$ or placebo: A pilot randomized controlled trial

We randomized 9 subjects to receive D_3, $1,25(OH)_2D$, or placebo. One individual in the $1,25(OH)_2D$ arm and one in the D_3 arm each were lost to follow up (Figure 6.1) and the ABP monitor failed to obtain blood pressure measures for one individual in the placebo group. We were therefore left with 7 subjects in the study for blood and urine markers and 6 subjects with blood pressure measures. All study subjects were using blood pressure lowering medications (Table 6.2). The majority of subjects were using a diuretic and either a calcium channel blocker or an ACE inhibitor. The three groups had different initial blood pressure and vitamin D levels (ANOVA $p<0.01$) but were balanced in terms of starting plasma renin activity, age, and use of medication. All subjects were black

Blood pressure changes

Systolic blood pressure decreased significantly in both the placebo and $1,25(OH)_2D$ groups but not in the vitamin D_3 group. The $1,25(OH)_2D$ group experienced a 13% reduction in systolic blood pressure while the placebo group only decreased by 4% ($p<0.001$ comparing change in placebo to change in $1,25(OH)_2D$). In subjects

randomized to calcitriol, there were no significant reductions in diastolic blood pressure; however, there was a significant reduction in heart rate (85 ± 9.3 vs 78 ± 7.6, p=0.004). The reduction in heart rate observed in the $1,25(OH)_2D$ group was significant when compared with placebo group ($p<0.001$).

Biomarkers

We did not observe a significant change in any of the serum biomarkers. We tested glucose, calcium, parathyroid hormone, creatinine, phosphorous, cholesterol, and triglycerides. All remained unchanged throughout the course of the study. Urinary excretion of sodium was not different across the three groups. Calcium excretion increased significantly in the group supplemented with $1,25(OH)_2D$.

$1,25(OH)_2D$ follow-up

We further evaluated whether the decrease in SBP was sustained after 1 week of $1,25(OH)_2D$ therapy. The subjects randomized to $1,25(OH)_2D$ were asked to wear the blood pressure monitor one week after conclusion of treatment (Figure 6.1). One week after subjects discontinued use of $1,25(OH)_2D$, systolic blood pressure returned to pre-treatment levels.

Pooled analysis – plasma renin activity

We pooled the subjects in our pilot study with those in the main study to examine the effect on blood pressure. Some of the subjects responded with a reduction in blood pressure while others did not (Figure 6.3). Only one subject randomized to vitamin D_3 experienced a significant reduction in blood pressure when compared with the placebo.

In this subject, plasma renin activity prior to vitamin D therapy was 2.4 ng/mL/hour which decreased to 0.7 ng/mL/hour. This was the only subject with plasma renin activity above 0.7 ng/mL/hour.

Discussion

We have demonstrated the active form of vitamin D, $1,25(OH)_2D$, is effective in reducing blood pressure in two African Americans with uncontrolled hypertension. We did not see any response in those randomized to vitamin D_3. Several mechanisms have been proposed through which vitamin D may target blood pressure control. Vitamin D therapy, including vitamin D_3, $1,25(OH)_2D$, and vitamin D analogues, are effective in the reduction of blood pressure (9, 13, 14), left ventricular mass (15, 16), albuminuria (17, 18). Further, vitamin D has anti-proliferative effects on vascular smooth muscle cells (19-21), reduces inflammatory cytokines (22, 23), and renin levels (10).

$1,25(OH)_2D$ therapy significantly reduced blood pressure in the present study. This finding is consistent with animal studies in which $1,25(OH)_2D$ reverses hypertension in mice depleted of $1,25(OH)_2D$ (10, 12) and 1-alpha hydroxylase knock-out mice (11). These findings are also supported by one human case study in which a Japanese man with high plasma renin activity went from 145/96 mmHg to 128/85 after two weeks of $1,25(OH)_2D$ therapy (24). $1,25(OH)_2D$ may have differential effects dependent upon both the individual and time after administration of dose. Short term administration of $1,25(OH)_2D$ increased peripheral resistance while decreasing cardiac output in hypertensive but not normotensive men (25). In a supporting animal model, rats given an injection of $1,25(OH)_2D$ showed an increase in arterial contractile force without an increase in blood pressure (26). Therefore, renin may not be the only blood pressure

regulation target for $1,25(OH)_2D$. The vascular endothelium itself may be a $1,25(OH)_2D$ target (19-21).

In addition to direct effects on the vascular endothelium and the RAS, $1,25(OH)_2D$ has been shown to reduce inflammatory cytokines from activated T-cells (22,23). Activated T-cells have been shown to increase oxidative stress and subsequently increase angiotensin-II production (27-29). Ang-II is a potent vasodilator and the downstream product of the RAS. Therefore one additional potential mechanism through which vitamin D may lower blood pressure is through reduction in inflammation leading to decreased RAS activation.

In addition to $1,25(OH)_2D$, active vitamin D, we have observed differential effect of vitamin D_3 therapy. In one individual with high renin hypertension, vitamin D_3 therapy successfully lowered both systolic and diastolic blood pressure. This effect was not observed in subjects with normal plasma renin activity. Others have suggested vitamin D may provide the greatest benefit in those with high plasma renin activity (30-32). Consequently, there may be populations more prone to the deleterious effects of vitamin D deficiency on hypertension.

Previous randomized clinical trials have evaluated vitamin D_3 as anti-hypertensive agent with mixed results (9, 13-15). A randomized, placebo controlled trial supplementing 145 elderly women with 400 IU vitamin D_3 and 600 mg calcium found systolic blood pressure decreased by 9.3% after eight weeks of supplementation while the placebo group decreased only 4% (9). A second study in the UK conducted in the winter found that supplementing 189 elderly people with one dose of 100,000 IU of vitamin D_3 decreased mean heart rate from 74 to 72 beats per minute but did not lower blood

pressure (15). However, serum 25(OH)D levels were only raised from 35 to 52 nmol/L which may not be sufficient to decrease in blood pressure. This clinical trial was repeated in Type 2 diabetics and blood pressure decreased by 14 mmHg when compared with placebo and endothelial function improved (13).

In addition to the potential for vitamin D as a treatment strategy, it may also be an effective prevention strategy. Prospective studies have demonstrated that individuals with low circulating 25(OH)D levels are at greatest risk of developing hypertension (7, 33). Further, low 25(OH)D levels are associated with a two fold increase in all cause mortality and cardiovascular mortality after a median follow-up of 7.7 years (34). Therefore, aggressively treating low vitamin D levels could reduce the burden of hypertension.

African Americans have a greater risk of hypertension, stroke death, and progression to end stage renal disease (3, 4, 35, 36). In addition they have much lower levels of circulating vitamin D levels (6, 37). Yet, none of the interventional studies examining vitamin D and cardiovascular health have included significant numbers of blacks. We have demonstrated that the benefit of vitamin D on blood pressure is not limited to only white subjects.

Our study was limited because we only had ten subjects complete all study related protocol. We were underpowered to examine the effects of D_3. However, we did observe significant reduction in blood pressure in one individual using D_3 with high plasma renin activity. We were not able to double blind the investigators because the dosing was different for the $1,25(OH)_2D$ and the D_3. However, we were able to blind the statistician conducting the analyses and subjects were blinded. Strengths include accurate

assessment of compliance with a supervised dose, demonstration of benefit in non-white subjects and those already using anti-hypertensive medication, and the use of 24 hour blood pressure monitoring to determine blood pressure.

Conclusion

Evidence exists indicating vitamin D therapy may be a complementary treatment strategy in the management of cardiovascular and renal disease. We have demonstrated in a small pilot study that blood pressure can be reduced with vitamin D therapy. Large scale studies examining the role of vitamin D in African Americans seem warranted.

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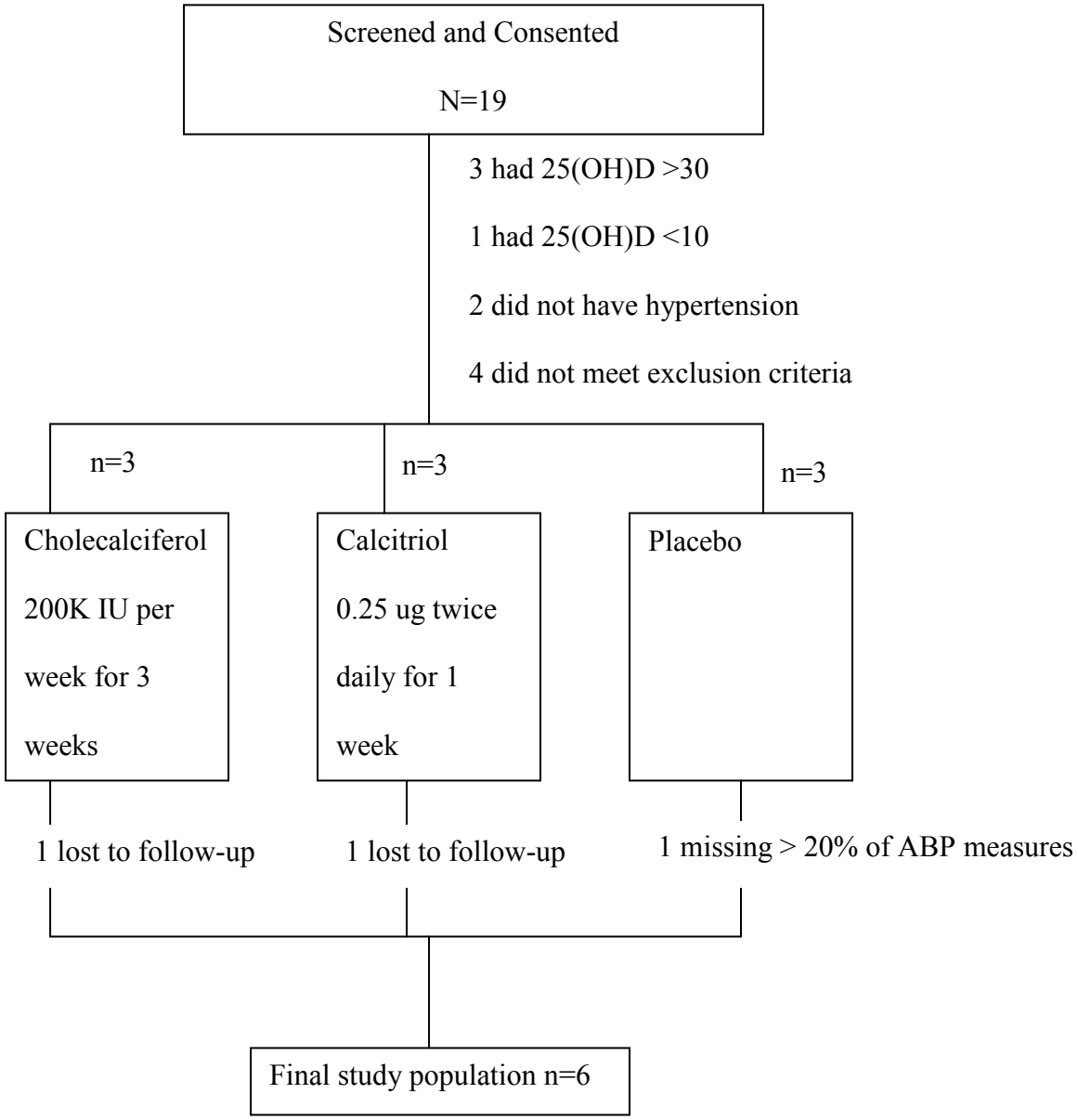
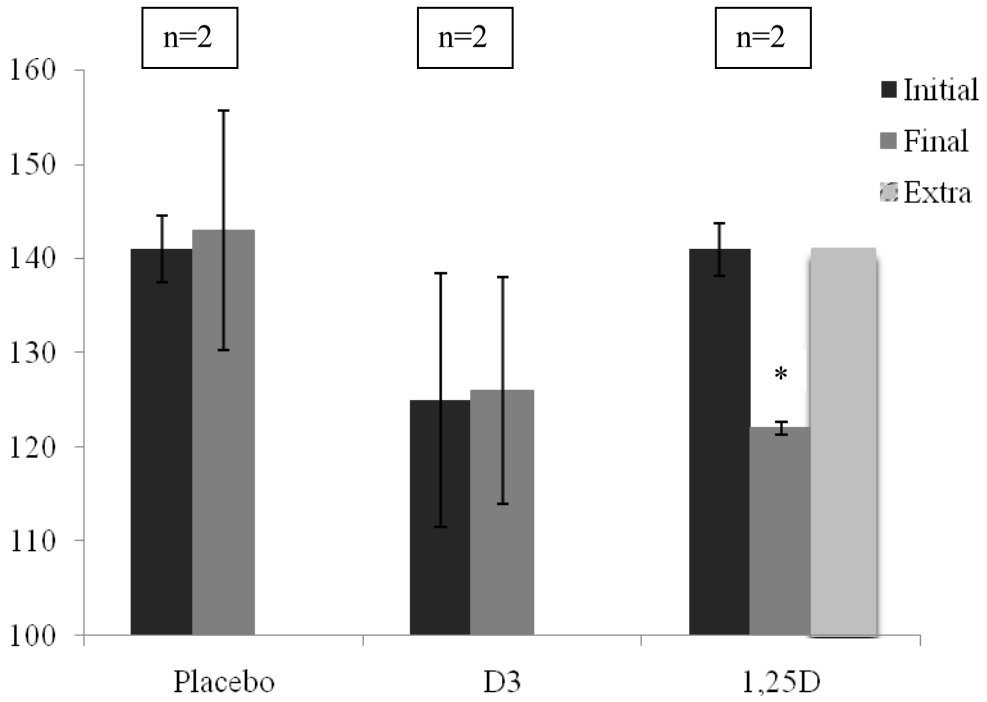


Figure 6.1 Subject recruitment and randomization



* $p<0.05$ when compared to initial and extra measure

Figure 6.2 Average 24 hour systolic blood pressure measures in three treatment groups. Blood pressure decreased significantly in those subjects receiving 1,25D when compared with D_3 and placebo

Table 6.1 Results pilot dosing study

	Age	Sex	Race	24 hour SBP* Initial	24 hour SBP* Final	Daytime SBP* Initial	Daytime SBP* Final	25(OH)D Initial (nmol/L)	25(OH)D Final (nmol/L)
Placebo	40	F	Black	128 ± 11.4	125 ± 8.7	130 ± 11.7	125 ± 8.7 [a]	30	47
D_3	54	M	Asian	148 ± 17.6	140 ± 14.8[a]	153 ±13.1	139 ± 8.6 [a]	6.2	57.6
D_3	42	F	Black	117 ± 11.9	111 ±12.4	122 ± 9.4	112 ± 12.1 [a]	31.4	95.9
D_3	47	F	Black	144 ± 10.1	137 ± 13.8 [a]	143 ± 10.7 [a]	135 ± 14.7 [a]	53.7	149.1
			Summary for D_3	136 ± 16.9	129 ± 15.9 [b]	139 ± 15.8	129 ± 14.6 [c]	30	101 [d]

*SBP – systolic blood pressure in mmHg

[a] indicates that $p<0.01$ when comparing average initial and final measures within one subject

[b] indicates that change in SBP when compared with placebo $p<0.01$

[c] indicates that change in SBP when compared with placebo $p=0.20$

[d] indicates that change in SBP when compared with placebo $p<0.01$

Table 6.2 Baseline characteristics

	Placebo	Calcitriol	D_3	p
n	3	3	3	
Age	46.7	46.5	42.3	0.67
Number of anti-HT medications	1.7	2.5	1.5	0.83
Blood pressure	141/84	141/76	125/70	0.02
Vitamin D (nmol/dL)	27	46	24	0.05
Plasma renin activity (ng/mL/hr)	0.5	0.7	0.7	0.35
Urinary CaCR (mg/mg)	0.1	0.1	0.04	0.28
Urinary ACR (mg/g)	45.8	57.9	48.9	0.33

Table 6.3 Blood pressure and serum biomarkers before and after intervention

		Placebo	D_3	**Calcitriol ($1\alpha,25(OH)_2D$)**
SBP	Initial	143 ± 11.6	125 ± 12.6	141 ± 17.8
(mmHg)	Final	137 ± 10.2	127 ± 17.2	122 ± 12.1
	p	0.016	0.55	<0.001
DBP	Initial	86 ± 10.4	72 ± 11.7	72 ± 11.7
(mmHg)	Final	86 ± 9.6	69 ± 9.2	69 ± 10.9
	p	0.84	0.20	0.23
Heart rate	Initial	88 ± 8.2	70 ± 11.7	85 ± 9.3
(bpm)	Final	90 ± 6.8	67 ± 9.2	78 ± 7.6
	p	0.30	0.20	0.004
Creatinine	Initial	0.73 ± 0.4	0.90 ± 0.2	0.75 ± 1.8
	Final	0.77 ± 0.4	1.00 ± 0.8	0.75 ± 0.4
Glucose	Initial	163 ± 2.1	118 ± 5.7	118 ± 4.2
	Final	144 ± 10.1	95 ± 3.5	123 ± 14.8
Serum Calcium	Initial	9.2 ± 0.02	9.4 ± 0	9.3 ± 0.01
	Final	9.3 ± 0	9.4 ± 0.01	9.3 ± 0.01
PTH	Initial	63	40	56
	Final	52	47	31
uCaCr	Initial	0.13 ± 0.06	0.08 ± 0.09	0.10 ± 0.03
	Final	0.13 ±0.06	0.07 ± 0.08	**0.20 ± 0.01**

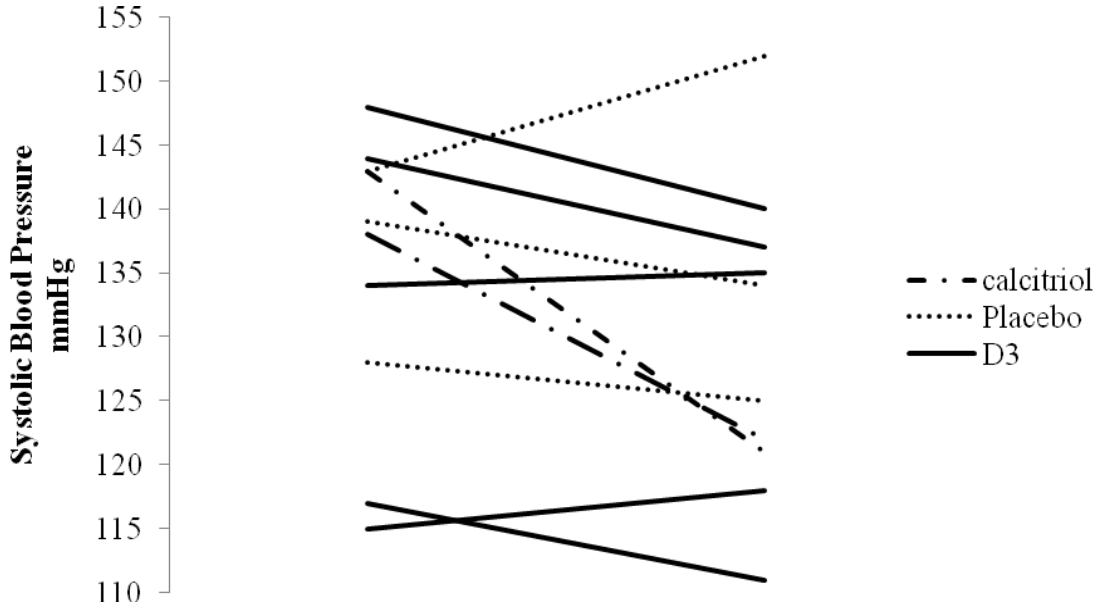


Figure 6.3 Pooled analyses from pilot trial and main study. Calcitriol group experience the biggest drop. Decreased in blood pressure in D_3 group was dependent upon starting blood pressure.

CHAPTER 7 EXPANDED METHODS

NHANES Analyses

Sample population

At the time of publication of Chapter 3 we were limited to NHANES III (1988-2004) because that was the only population with 25(OH)D levels. Data from NHANES III represent the non-institutionalized United States population ≥ 2 months of age and were collected by the National Center for Health Statistics, Centers for Disease Control and Prevention (CDC) using field clinic visits at a mobile examination center and household interviews. The survey was approved by the CDC Institutional Review Board and written consent was received from participants. After being interviewed in their home, participants were invited to attend one of three physical examination sessions. Blood pressure was checked at both the in-home interview and in the mobile examination center. Blood was drawn only at the mobile exam center. Blood samples were centrifuged and serum aliquoted, and frozen to -70°C on site during examination and then shipped on dry ice to central laboratories where they were stored at -70°C until analysis. 25(OH)D was measured using a radioimmunoassay (RIA) kit from DiaSorin with a lower limit of detection of 8 ng/ml.

Reliable 25(OH)D concentrations and systolic blood pressure measures were available on 16,135 participants aged ≧19 years. We pooled both phase I and phase II together for analysis. We excluded Hispanics, those using anti-hypertensive medication,

and those told by their doctor that they had hypertension which left a sample population of 7699.

Blood pressure determination and categorization

We used the blood pressure measures from the mobile examination center because they were obtained at the same time as the blood draw. Three sets of blood pressure measurements were taken in the examination center by trained personnel. The average of the three measures was used for analysis. Blood pressure measurements were taken using a mercury sphygmomanometer (W. A. Baum Co., Inc, Copiague, NY) according to the standardized blood pressure measurement protocols recommended by the American Heart Association. Blood pressure was sub-divided into 6 categories based on the recommendations from the Joint National Committee 7 (<120 mmHg-Normotensive, 120-129 mmHg - Pre-hypertensive, 130-139 mmHg – Borderline, 140-159 mmHg - Stage I Hypertension, and >=160 mmHg - Stage 2 Hypertension). JNC 7 classifies normotensives as <120 mmHg; however, we broke this category into two separate groups (<110 mmHg-Normotensive and 110-119 mmHg - High normal) to create the sixth category.

Statistical analysis

We tested all continuous variables to insure normality. Univariate analyses were conducted using PROC DESCRIPT and multivariate linear regression was conducted using PROC REGRESS in SAS callable SUDAAN 9.0 (SAS Institute, Cary, NC). We tested age as a continuous and categorical variable (two different cut-points 19-49/ 50+

and 1—39/40-59/60+), race, vitamin D status (deficient, insufficient, sufficient), presence of diabetes, and level of hypertension (JNC definitions) for interaction. We considered any interaction $p < 0.10$ to be significant. Age, race, level of hypertension, and vitamin D status were all found to be significant effect modifiers. We therefore performed four separate stratified analyses. We tested age, sex, presence of diabetes, physical activity, smoking status, season of blood draw, latitude in which participant was dwelling at the time of sampling, vitamin D intake, calcium intake, and race as potential confounders. In order to protect the privacy of certain subjects in the sample, latitude information was only available for 46% of the subjects.

After we assembled our tables, we performed sensitivity analyses to ensure the robustness of our analysis. We ran our models including Hispanics and found a similar association of vitamin D with blood pressure. We also included all subjects in the data set including those with hypertension and using anti-hypertensive medication. Although use of an anti-hypertensive medication was an effect modifier, the association between blood pressure and 25(OH)D was not present in those using medication due to the small number of subjects in each cell after stratifying by age, race and 25(OH) status.

Hypertension Clinical Trial

Identification of study population

In order to define our sample population and adequately power our clinical study, we conducted an analysis using NHANES III (1988-1994) to determine if there was an association between circulating 25(OH)D levels and blood pressure (Chapter 3). We used data to determine inclusion criteria for our study population. Upon publishing the paper and before beginning clinical trial, we had more recent data from NHANES 2002-2006 demonstrating a strong association with 25(OH)D and blood pressure in African Americans. We used similar methods as described in Chapter 3 to regress 25(OH)D with systolic blood pressure. We used both hypertensives and normotensives for this table. Medication use was not an effect modifier nor was age in the NHANES 2002-2005 analysis. We therefore used African Americans as our primary study population and allowed subjects to be included in our study regardless of medication use. This was done not only because of the NHANES results but also because of the significant burden of hypertension and vitamin D deficiency in blacks.

Dose identification

In order to determine the dosing strategy, we conducted a retrospective chart review of 306 consecutive patients who were prescribed ergocalciferol (vitamin D_2) at the Atlanta Veterans Administration Medical Center (VAMC) between February 2003 and May 2006. This study was approved by the Emory University Institutional Review Board and Veteran's Affairs Research and Development Committee. Descriptive

statistics were used for demographic and laboratory data. To compare differences by ANOVA in the vitamin D repletion regimens, we used PROC GLM in SAS version 9.1 (SAS Institute, Cary, NC) to produce least square means.

We found 63 discrete modalities of vitamin D repletion regimen. Cholecalciferol is not prescribed at the VAMC so we examined ergocalciferol prescribing patterns. The prescribed dose of ergocalciferol ranged from 400 IU to 50,000 IU, and frequency of dosing ranged from twice daily to once monthly. We did not observe significant increases in vitamin D based on dosing modality (daily, weekly, monthly).

We evaluated the effects of total ergocalciferol dose on changes in 25(OH)D, PTH, and calcium. The patients were divided into three groups based on total IU of ergocalciferol: < 300,000 IU; 300,001-600,000 IU; and >600,000 IU. All total doses of vitamin D increased 25(OH)D significantly ($p < 0.01$). Vitamin D sufficiency was most frequently achieved when patients received > 600,000 IU (66%). None of the subjects had hypercalcemia; therefore we used a total dose of 600,000 IU.

We tested a total dose of 600,000 IU in a pilot study by giving a weekly dose of 50,000 IU for 12 weeks. We enrolled subjects and randomized them to receive either placebo or vitamin D_3. Four subjects received vitamin D_3 and two received placebo. One of the subjects receiving placebo refused to come back in for follow-up and one subject receiving vitamin D_3 refused to give a blood sample on follow-up because he had not completed medication. We were therefore left with four subjects in this pilot study to confirm the dose.

We had two subjects fail to complete study protocol over the 12 weeks. Therefore we decided to compress and supervise the dosage to improve compliance.

From the original chart review, we learned the length of time the dose was administered and dosing modality were not significant predictors of attaining vitamin D sufficiency. We therefore provided a total dose of 600,000 IU of vitamin D given over 3 weeks (200,000 IU per week) for the vitamin D_3 arm.

Overview

We studied the effect of two forms of vitamin D on blood pressure. The single blinded randomized clinical trial was conducted between July 2006 and July 2008. Subjects were recruited based on presence of hypertension and vitamin D insufficiency from Veteran's Affairs Medical Center (VAMC) and outpatient clinics in metropolitan Atlanta, GA. Subjects were given either supervised doses of 200,000 IU vitamin D_3 or placebo pills to be taken weekly over 3 weeks or were given 0.5 ug $1,25(OH)_2D$ to be taken twice daily for one week. Blood was collected to test serum 25(OH)D, PTH, 1α,25(OH)2D, ang-II, aldosterone, and rennin. In addition subjects wore a blood pressure monitor for 24 hours to obtain average 24 hour and waking blood pressure. This study was approved by the Emory University Institutional Review Board and Veteran's Affairs Research and Development Committee. The study is registered at clinicaltrials.gov under study number NCT00459563.

Study population

Subjects were recruited from both the Veteran's Administration Medical Center (VAMC) and Emory communities. To recruit subjects we worked with primary care clinics (satellite clinics) at the VAMC, conducted health fairs, posted signs at Emory and

the VAMC, and utilized internet and print advertising. We held four health fairs over the two years which generated a list of 200 potential subjects. We attended regular hypertension education classes every Friday at the outpatient clinic in Decatur, GA to inform subjects about the study. We submitted advertising to Craig's List, clinicaltrials.gov, the Emory Wheel and posted signs throughout the VAMC and Emory communities.

Eligibility

In order to be eligible for the study, subjects had to be over the age of 30, self-define as black or African American, have vitamin D levels between 10 and 30 ng/mL, and have systolic blood pressure between 130 and 150 mmHg. Subjects were excluded if they exhibited hypercalciuria, hyperparathyroidism, current use of more than three anti-hypertensive medications, inability to understand the consent form, inability to return blood pressure monitor within 24-48 hours after visit, history of alcohol abuse, current alcohol consumption of greater than two drinks per day, diagnosis of chronic kidney disease, diagnosis of HIV, history of heart disease, history of stroke, inability to comply with study protocol, current treatment for cancer, narcotic dependence, current use of vitamin D analog, and current use of greater than 2000 IU of vitamin D. In addition subjects were excluded if they did not show up for more than three screening visit and/or baseline visit appointments because this demonstrated an inability to comply with study protocol.

Study medication

Subjects received $1,25(OH)_2D$ manufactured by Pliva Inc. (East Hanover, NJ) vitamin D_3 or placebo manufactured by Tishcon (Westbury, NY). Vitamin D_3 and placebo were taken orally and are identical in shape and color. Study personnel observed subjects taking vitamin D_3 and placebo during the visits to ensure compliance with study medication. $1,25(OH)_2D$ had to be taken twice daily for one week so subjects took medication the first and last pill in the presence of study personnel but took all other pills at home. Subjects brought bottles containing $1,25(OH)_2D$ with them to the final visit to assess compliance with medication. Medication was stored in the VAMC research pharmacy. Bottles were randomized prior to delivery to the pharmacy.

Randomization and blinding

Subjects were randomized at baseline visit. Study personnel were blinded to vitamin D_3 and placebo as were subjects; however, due to differences in dosing regimen study personnel were not blinded if subject was randomized to $1,25(OH)_2D$. Subjects were blinded to study drug but were told at the conclusion of the study which drug they were receiving. If a subject would have requested to know which medication he/she was receiving, the VAMC pharmacy technician had a master unblinded list and could have informed subject. This did not happen throughout the course of the study.

Safety

There were no adverse outcomes reported in this study. Subjects were closely monitored to ensure safety. Subjects had contact with study personnel once per week and

could report adverse outcomes at anytime 24 hours a day. They were given an emergency contact number to get in touch with investigators. In addition they were given the phone number for the IRB and the Research Compliance Officer at the VAMC Clinical Study Center should they need to report any safety concerns or adverse events and did not feel comfortable contacting study personnel. We also monitored urine levels weekly for potential hypercalcemia.

Consent and screening

Subjects were consented at the screening visit. At the screening visit, subjects provided 10 mL of blood to test 25(OH) D levels, answered a brief questionnaire to determine eligibility, and had blood pressure measured. To ensure subject meets blood pressure requirements, the patient sat quietly in the examination room for 5 minutes. Study personnel obtained blood pressure using an automated blood pressure cuff validated and maintained by the VAMC Clinical Studies Center. This one time measure was only used for screening purposes. To ensure subjects had 25(OH)D within the target range (10-30 ng/mL), study personnel used an ELISA kit (IDS Ltd, Fountain Hills, AZ, detection limit: 6-360 nmol/l (2.4-144 ng/mL), product number: AC-57F1). The results have been validated by a D binding assay conducted by Dr. Holick's laboratory in Boston in which the r^2 was 0.68. Screening visit 25(OH)D was obtained within one week of screening visit. This one time measure was only used for screening purposes.

Baseline visit

Subjects had blood pressure measured to ensure they were still within the target range for the study. If so, they were randomized and received study medication. Subjects wore a 24 hour ABP monitor and returned the monitor the following day. While subject wore the monitor, he/she collected 24 hours of urine to be returned with monitor. The urine was to assess potential hypercalcuria (using calcium-creatinine ratio) and any dietary sodium changes. Subjects were instructed to continue using any medications they were using prior to study start and to limit sunlight exposure or wear at least SPF 15. If subject was randomized to $1,25(OH)_2D$ a follow-up visit was scheduled for one week later to finalize study participation. If subject randomized to either placebo or vitamin D_3, subject received study drug after wearing blood pressure monitor. In addition, a visit was scheduled for one week, two weeks, and three weeks later. The first two visits were for study drug administration and the last was to finalize study participation.

Final visit

Subjects had blood drawn and repeated the 24 hour blood pressure monitoring and urine collection. Study personnel interviewed subjects to ascertain if they had any adverse events or unpleasant side effects while receiving the study medication. If they received $1,25(OH)_2D$, they returned pill bottle and remaining pills were counted. After subjects returned the blood pressure monitor, they were given a piece of paper indicating which treatment they were receiving and instructed not to inform study personnel which medication was received, in order to keep the investigators blinded.

Blood Collection

Blood was collected at the screening visit, baseline, and final visits. Blood was drawn by a certified phlebotomist working in the VAMC laboratory. During baseline and follow-up blood draw, three tubes of blood were drawn (corresponding to about 30 mL blood): a 5 mL tube with a red top was sent to the VAMC laboratory to analyze serum electrolytes, a 10 mL red top tube, 10 mL green to tube, and 10 mL purple top tube were spun down in a -5C centrifuge. The respective serum or plasma was alloquoted into seven 1.2 mL labeled cryovials and stored in a -70C freezer until they were needed for analysis. Subjects were informed in the consent that blood would be stored for future testing and were provided a revocation letter should an individual require his/her samples be destroyed at a future date. Protocol for collecting and storing blood specimens were verified with the GCRC to ensure proper handling of samples.

Quality Assurance

We compared blood levels of 25(OH)D at the beginning and the end of the study. Baseline and follow-up samples from each patient were tested in the same batch to ensure that within test variability was minimized within each patient. In order to ensure accuracy of each batch of testing, a standard sample of known concentration was run with each batch. Renin levels were determined by Quest Diagnostics in San Jose, CA. Urinary creatinine, potassium, calcium and sodium levels were measured by the VAMC laboratory using standard methods.

Blood Pressure Monitor

Subjects were requested to wear an ambulatory blood pressure (ABP) monitor made by Spacelabs (Spacelabs 90207) for 24 hours. The Spacelabs 90207 is a validated 24 hour ABP monitor and is commonly used in studies. Study personnel measured subject's blood pressure using a validated automated blood pressure machine 5 minutes after he/she has been seated quietly. Study personnel then placed the ABP subjects arm and instruct the subject on how to operate the device. The first reading from the ABP monitor was checked with the measure obtained using Clinical Studies Center blood pressure machine to ensure that the monitor wad working properly. The monitor and seated measure were within 10 mmHg in all subjects. When the subject returned 24 hours later the same process was used. The Spacelabs 90207 comes with software for downloading blood pressure measures collected by the monitor. Subject blood pressure measures were downloaded as soon as the monitor was returned so that the monitor is ready for the next subject. Monitor was initialized for each subject within 24 hours of subject appointment. The blood pressure cuff inflated every 15 minutes during the day time and every 30 minutes at night.

Statistical Analysis

Data was analyzed using mixed linear models to account for correlation of repeated measures within the same subject. We used Microsoft Excel and SAS 9.1 (SAS Institute, Cary, NC) for all data analyses. We used ABP monitors to minimize measurement bias. Data from the ABP monitors was downloaded using software provided by the manufacturer and was then transferred to Excel using an encrypted

spreadsheet. Blood pressure averages were obtained using Microsoft Excel and then transferred into a SAS data set.

Using an ambulatory monitor helps to reduce measurement bias since multiple measures are taken for one individual. Since repeated measures were present we performed two sets of analyses. In the first set we considered all measures for an individual to contribute to the overall variability. We used PROC MIXED to identify a mean and standard error for each set of blood pressure measures within an individual. The intercept was considered to be the mean value and was the conditional means model for determining average blood pressure within in individual. We used these means to test for significant changes between baseline and follow-up visit. We used maximum likelihood as method of estimation. We examined the variance-covariance matrix structure to determine how much variability the individual contributed to the model as well as how much variability the treatment assigned contributed to the model.

In the second set of analyses, we considered the average calculated by excel to be one data point and used this data point as a summary measure. This mean blood pressure was then averaged with other mean blood pressures and summary statistics were used to compare group changes in blood pressure.

After univariate analyses, data was analyzed using PROC MIXED with a REPEATED statement to account for repeated measures within the same subject. We also used PROC GLM to calculate least squared means controlling for initial blood pressure and used a Bonferroni correction. This method also enables a more accurate calculation of standard error as it has the ability to account for both intra and inter-individual variability. Average waking blood pressure was used as the main outcome

variable. Changes in blood pressure (from baseline to follow-up) in the treatment group were subtracted from those in the placebo group to account for regression toward the mean. We also examined changes in biomarkers between treatment and control groups and examined ratio of daytime to nighttime blood pressure changes. This measure is called dipping in ABP studies. All analysis was done using intent to treat.

Subject Confidentiality and Privacy

Subjects signed a VAMC HIPPA form at the time of consent explaining their right to privacy. We indicated in the subject computerized patient record system (CPRS) at the VAMC that subjects were participating in a clinical study to inform physicians caring for subjects. Demographic and dietary information was collected from subjects in addition to serum, plasma, and blood pressure measurements. Subjects were de-identified in all data sets and on all sample vials by using a study identification number that can not be traced back to a specific subject. Subject identification number was contained in subject folder which is kept in a locked filing cabinet in a locked laboratory at the VAMC for three years after subject has completed the study. At that point subject folders maintained in the VAMC laboratory will be shredded to maintain privacy of subject. At the beginning of the study subjects were giving a letter and a stamped envelope addressed to study personnel to opt-out of the study at any time. This ensures voluntary participation in study related activities.

Benefits to the Subjects

Subjects received a book published by the American Heart Association on how to control blood pressure. In addition subjects were compensated with $180 total for participation. Subjects were compensated with $20 at all visits except the visits when monitor and urine were returned when they were compensated $40. Subjects were compensated with cash at the time of the appointment. Initially a check based system was used but subjects were unhappy with the amount of time it took to receive payment.

Study Design - Vitamin D Repletion in Cystic Fibrosis Patients

Background

To examine the study population proposed for the clinical trial, we conducted a retrospective chart review using patient data at the Emory Cystic Fibrosis Center from 2004 and 2005 (Chapter 7). This study was approved by the Emory University Institutional Review Board. Serum 25-hydroxyvitamin D (25(OH)D) concentrations were determined in 156 patients over the two year period indicating that 14.8% of patients did not have 25(OH)D levels checked during that time period. Further, we found that 76% of subject had vitamin D insufficiency. We therefore determined we would be able to find enough subjects using only the Emory Cystic Fibrosis Center.

Overview

We sought to examine the best way to replete 25(OH)D in persons with Cystic Fibrosis. We had three arms: ergocalciferol (D_2) cholecalciferol (D_3), and UV light box. Subjects were followed for 12 weeks in the winter and early spring to determine which method was best for raising 25(OH)D levels. The study was approved by the Emory Institutional Review board (IRB) and all subjects gave written informed consent. The study was registered at clinicaltrials.gov under study number NCT00450073.

Study population

Subjects with CF were recruited from the Emory Cystic Fibrosis Center from November of 2006 to March of 2008. The timing of recruitment was limited to the late fall and winter months in order to minimize the contribution of endogenous vitamin D_3 production from the sun. The staff at the Emory CF Center aided study personnel in identifying interested subjects. Study personnel attended clinic at least once per week for screening of subjects. If a potential subjects indicated interest in participating in the study, they were provided with an informed consent and evaluated for initial eligibility.

Eligibility

Patients were included in the study if they were between the ages of 16 to 70, had a screening 25(OH)D level between 10 and 40 ng/ml, and had a confirmed diagnosis of CF by genetic analysis or sweat chloride testing. The confirmed diagnosis of CF was obtained from patient chart records. Exclusion criteria were history of skin disease or cancer, presence of renal or hepatic disease, recent treatment with high dose vitamin D, treatment with prednisone, or a history of more than 6 hospitalizations within the past year. Patients were also excluded if they were planning any trips during the study to sun-rich areas lasting longer than 2-3 days.

Study medication

We did not use a placebo in this study because it would not be ethical to withhold treatment of vitamin D deficiency in this population. The vitamin D_2 50,000 IU and vitamin D_3 50,000 IU capsules were obtained from Pliva, Inc. (East Hanover, NJ) and

Tishcon Corp. (Westbury, NY), respectively. A sample of each vitamin D capsule was assayed by an independent laboratory using high-performance liquid chromatography (HPLC) with a reversed phase column. The vitamin D_3 tablets were found to contain 49300 (+/- 900) IU of vitamin D_3 and the vitamin D_2 gel capsules were found to contain 47100 (+/- 1100) IU of vitamin D_2.

UV light therapy exposed their lower backs in a seated position to a portable UV indoor tanning lamp (Sperti sunlamp "Del Sol", Crescent Springs, KY) at a distance of 14 inches for 3-10 min depending on their skin type five times a week according to a UV light protocol developed to enhance vitamin D status in CF patients. The Sperti sunlamp mimics natural sunlight in that it emits 2-5% UVB between the wavelengths of 290 to 320 nm. Subjects were contacted on a weekly basis to ensure compliance with their respective treatment regimens.

Randomization and blinding

Thirty CF subjects were randomized in blocks of 6 to three treatment arms: vitamin D_3, vitamin D_2 or UV light therapy. D_2 and D_3 pills were not similar in color or size. A treatment was drawn out of an envelope and that treatment was given to subjects. Since it was obvious which treatment was given to the subjects, it was not possible to use double blinding or even single blinding.

Baseline visit

Consent was obtained at baseline visit. Serum was collected for 25(OH)D and parathyroid hormone (PTH). We recorded most recent FEV1 percentage in the subject

record. Mean daily intake of vitamin D by subjects was estimated using a food frequency questionnaire. Subjects were deemed to have vitamin D insufficiency if a 25(OH)D level was less than 40 ng/mL at anytime within the preceding 12 months.. If any subject had 25(OH)D levels greater than 40 ng/mL we contacted that individual and informed him/her to discontinue medication use. We had historical 25(OH)D levels in the chart to use as a guide and we confirmed 25(OH)D levels in our lab within two weeks of randomization. We interviewed the subjects to determine what type of skin he/she had based on susceptibility to burns and ease of tanning.

Final Visit

Serum was collected for 25(OH)D and parathyroid hormone (PTH). We recorded most recent FEV1 percentage in the subject record. Mean daily intake of vitamin D by subjects was estimated using a food frequency questionnaire. All subjects returned to the CF center within 4 weeks of finishing last study related medication.

Blood collection

Blood was collected at baseline and final visits. Blood was drawn by study personnel or nursing staff at the CF center. During baseline and follow-up blood draw, two tubes of blood were drawn (corresponding to about 15 mL blood): a 10 mL tube with a tiger top and a 10 mL purple top tube were spun down in a room temperature centrifuge. The respective serum or plasma was alloquoted into 1.2 mL labeled cryovials and stored in a -70C freezer until they were needed for analysis.

Aliquots of serum were sent to Quest Diagnostics Incorporated (San Juan Capistrano, CA) for measurement of $25(OH)D_2$, $25(OH)D_3$, and total 25(OH)D using LC-MS. PTH was done using ELISA (Immutopics International, LLC, San Clemente, CA).

Statistical analysis

Data was first examined to ensure normality. All change variables were normally distributed. We used SAS 9.2 (SAS Institute Cary, NC) to perform all analyses. The study was originally powered to detect a difference of 5 ng/mL. We used Fisher's Exact Test (PROC FREQ) to calculate p-value comparing count data because many of our cells had less than 5 subjects. Simple ANOVA was used to compare differences in mean covariates by treatment groups and ANACOVA to compare mean differences when controlling for covariates (PROC GLM). Least squares means were calculated for all means reported using a Bonferroni correction whenever repeated measures were present. To determine whether initial 25(OH)D status effected change in 25(OH)D we used Pearson's correlation (PROC CORR). When considering our final models, we used initial 25(OH)D status, race, age, type of CF mutation, serum calcium, vitamin D intake, use of vitamin supplement as potential covariates. We tested for interaction with race, CF mutation, and use of vitamins however significant interaction was not present.

Subject confidentiality and privacy

Subjects signed an Emory University HIPPA form to explain their rights to privacy. We informed the subjects primary care physician at the CF center that the

subject was participating in the clinical study to ensure that they would not be treated for vitamin D deficiency during study period. Demographic and dietary information was collected from subjects in addition to serum, plasma, and blood pressure measurements. We de-identified this information by assigning a patient ID that included subject initials and date of enrollment. We used this coding system on all cryovials. We later de-identified the samples further by removing the subject initials and recruitment date and assigned a subject ID number that could not be traced back to the subject. All subject records were kept in a locked filing cabinet in a locked room at the Veterans Affairs Medical Center. The locked room could only be accessed by individuals who had an access card to get to the laboratory.

Benefits to subjects

Subjects were compensated for time and travel to the CF center. We collected all mileage information and receipts for expenses incurred in route to the CF center and reimbursed subjects for those expenses.

CHAPTER 8 SUMMARY AND CONCLUSIONS

Key Findings

Vitamin D deficiency is common. In particular, vitamin D deficiency is epidemic in African Americans with hypertension and individuals with cystic fibrosis. This epidemic has received considerable attention in recent years. However, the increase in awareness has not translated to a reduction in vitamin D deficiency. Therefore, we examined the prevalence, consequences, and treatment of vitamin D deficiency. Since treatment of deficiency may vary based on overall health, we used hypertension and cystic fibrosis as model diseased populations to study vitamin D deficiency. This enabled us to study deficiency in the context of both genetic condition affecting a small portion of the population and a chronic condition affecting over half of the U.S. population.

Chapter 2 of this dissertation outlined the biology and epidemiology of vitamin D. It also described definitions of deficiency and strategies for repleting vitamin D levels. Further, chapter 2 explored deficiency as it relates to hypertension and cystic fibrosis. Subsequent chapters presented data suggesting that vitamin D deficiency is common in both populations, despite increased awareness. Further, current treatments are not working to improve vitamin D status to eliminate deficiency. This can be problematic in both populations. We therefore examined the best strategy to improve vitamin D status after quantifying the scope of the problem. This included two papers that have been accepted for publication and two papers are currently in review.

In Chapter 3, we demonstrated that more than 90% of African Americans have vitamin D deficiency. The high levels of vitamin D deficiency did not provide a suitable reference group when comparing vitamin D deficiency and hypertension in this racial sub-group. However, we were able to demonstrate significant association between blood pressure and vitamin D status in white individuals. Since we were unable to study large numbers of African Americans who had sufficient vitamin D levels, we designed a clinical trial to first restore vitamin D levels. We could then examine the effect of adequate vitamin D status on blood pressure.

When we tested two different forms of vitamin D (D_3 and $1,25(OH)_2D$) in African Americans (Chapter 6), subjects receiving the active form of vitamin D ($1,25(OH)_2D$) demonstrated a significant decrease in blood pressure compared with those receiving placebo. One week after cessation of $1,25(OH)_2D$ therapy blood pressure increased significantly to pretreatment levels. Those receiving vitamin D_3 did not have a similar decrease in blood pressure compared to placebo. However, sub-analysis combining data from a pilot study revealed vitamin D_3 therapy may be beneficial in those with high renin hypertension.

Although current recommendations for CF patients are to have yearly 25(OH)D levels checked and treated if necessary, we found that only 76% of patients had sufficient vitamin D levels and less than 10% have yearly 25(OH)D levels checked (Chapter 4). When examining strategies for correcting vitamin D deficiency (Chapter 5), we found that vitamin D_3 was the best treatment when compared with UV light and vitamin D_2 for raising 25(OH)D levels in CF patients. Further, we found that treatment with vitamin D_3

could result in sufficient vitamin D status in CF patients. Both vitamin D_2 and D_3 significantly reduced parathyroid hormone levels, but UV light did not.

Educational Opportunities

Currently most people being treated for deficiency are being seen by endocrinologists or nephrologists. Moving forward, treatment of vitamin D deficiency should move into primary care. However, this could be complicated due to a lack of understanding regarding diagnosing and treating of vitamin D deficiency.

There are three steps to helping the medical community understand the scope of vitamin D deficiency. First, is to explain the consequences of deficiency so doctors understand the importance of diagnosing the condition. In addition, doctors need to understand how to diagnose deficiency. The companies providing vitamin D assessment would be instrumental partners in this step. Second, is to increase patient understanding of vitamin D deficiency. Since patients are able to treat this condition from home, it is important they understand how much vitamin D is necessary and how they can obtain vitamin D. They also need to understand the importance of having 25(OH)D levels checked periodically. This could be done using massive media communications so that individuals could become educated about vitamin D and discuss it with their doctors. After patients and doctors are made aware of vitamin D deficiency and how to test for this condition, the next step is treating the disease. It is necessary provide doctors with the tools to treat deficiency that would be specific to the individual.

In addition to increasing awareness of vitamin D, it is it is critical to further explain vitamin D toxicity since this appears to be a misunderstood phenomenon. The first step in demystifying vitamin D toxicity is to provide information to patients and doctors explaining vitamin D physiology. This is very important as many people do not understand the differences between the various forms of vitamin D. Many people use the word vitamin D to mean analog therapy, calcitriol, 25(OH)D, and vitamin D itself. Therefore, providers may order the wrong test to check vitamin D status and may also provide the wrong treatment.

As we have demonstrated in this dissertation, the CF and hypertensive communities represent two unique opportunities for correcting vitamin D deficiency and improving health status. However, there is much that needs to be done to translate some of these findings to the communities at large.

In the CF community, it is recommended that vitamin D deficiency be aggressively assessed and treated. We have found this is not currently being done. Further, we demonstrated 50,000 IU weekly vitamin D_3 is an excellent therapy to improve vitamin D status in individuals with CF. Therefore, we recommend greater education for doctors treating CF patients to ensure they understand how to diagnose and treat vitamin D deficiency. The training materials could be presented at national meetings and distributed to CF centers to help in disseminating this recommendation. In addition, it would be beneficial to add vitamin D to the standard yearly panel of blood work done on CF patients. The training materials coupled with regular screening would have to eliminate vitamin D deficiency in the CF community.

In the hypertension community, there is not a global recommendation to treat vitamin D deficiency. One way to improve the visibility of vitamin D in treating hypertension is to work with the community of primary care doctors. Since most hypertensive patients will be identified in primary care medicine, it is critical this subset of physicians be trained in treatment of vitamin D deficiency. It is not likely the pharmaceutical companies will be involved in this process; therefore, the education will have to come from within the community of science. This is complicated because there will not be the same level of funding that would be available if there were drug companies funding the campaign. There are enough studies published at this time to suggest treatment of vitamin D deficiency will help resolve high blood pressure. This information should be summarized and distributed to primary care doctors. Information could be disseminated in medical journals, at national meetings, and through visits to local primary care clinics. In addition, vitamin D levels should be added as a standard test when an individual has newly diagnosed hypertension and should be included in any annual blood work done in hypertensive patients.

Policy Implications

A consensus statement on the management of vitamin D deficiency is currently needed. Vitamin D levels are not routinely checked outside of the nephrology and endocrinology communities. It is possible that some primary care physicians would not understand how to interpret vitamin D levels and how to properly order vitamin D levels.

Therefore, it is critical to provide materials to educate physicians on the importance of and methods for vitamin D screening. Checking vitamin D levels are not routine and therefore may not be covered by insurance companies. Informing the insurance providers of the potential cheap prophylactic benefits of vitamin D therapy could help to increase visibility. A partnership could be reached with Kaiser Permenante, the VAMC, or other large provider of health care to examine the benefits of vitamin D screening and treatment in the primary care setting.

Although screening of vitamin D deficiency is important, it is also critical doctors know how to treat deficiency should the condition be identified. The benefits of vitamin D_3 over other forms of vitamin D need to be communicated. It is not possible to obtain vitamin D_3 using a prescription and physicians need to be aware of this fact. It is not necessary to treat deficiency with a prescription as over the counter therapies are also highly effective.

Next Steps

A variety of clinical and animal studies are needed to confirm and expand upon the information presented in this thesis. Four main areas of study for hypertension should be considered: analog therapy, vitamin D_3 for the treatment of high renin activity, vitamin D and inflammation, and vitamin D as prophylaxis for hypertension in blacks. For individuals with cystic fibrosis, two main studies are needed: translation studies to

determine if vitamin D therapy improves health and determination of appropriate vitamin D therapy for acutely ill CF patients.

Although we have demonstrated a reduction in blood pressure using $1,25(OH)_2D$, this therapy cannot be recommended broadly due to the potential for hypercalcemia. First, studies considering non-calcemic analogs of $1,25(OH)_2D$ would be beneficial in determining the potential for these drugs as anti-hypertensive agents. There are two main drug companies producing vitamin D analogs that could be approached regarding funding for this type of research. Since one third of individuals in the U.S. have high blood pressure and require treatment, vitamin D analogs could potentially be novel as anti-hypertensive drugs.

Second, since we have observed in one subject with high renin activity the potential for vitamin D_3 therapy to reduce blood pressure, larger scale clinical trials to examine the impact of correcting vitamin D deficiency while better controlling renin levels seem warranted. This study should be done using a large clinical trial. The study should be adequately powered to stratify on plasma renin activity and also on salt sensitivity. Since it is possible that salt sensitivity could contribute to ability of vitamin D to interact with the renin angiotensin-II aldosterone system, it is important to control for this form of hypertension.

Third, the role of vitamin D in reduction of inflammation needs to be elucidated. Although it is known that hypertension results in an inflamed state and that vitamin D therapy may reduce inflammation, it is not know if this is a mechanism through which vitamin D reduces blood pressure. These studies could be conducted in two ways. First T cell cultures could be used to determine if varying levels of $1,25(OH)_2D$ in the media

results in reduction of inflammatory cytokines. We would then test to see if reduction in cytokines reduced activation of the RAS. This would help explore the vitamin D – inflammation – RAS pathway. Second, we could treat individuals with vitamin D deficiency and obtain T cells before and after treatment. Those cells could then be examined using microarray chips to determine if markers of inflammation and hypertension have been modified.

Fourth, longitudinal studies examining the association between vitamin D deficiency and incident hypertension in African Americans should be undertaken. This is necessary to determine if the associations observed in largely white cohorts can be reproduced mixed race populations. Although we have seen an association with hypertension and vitamin D in a small pilot study and in NHANES data, it is not known if the vitamin D deficiency occurs before hypertension. It could also be possible that the vitamin D deficiency is the result of hypertension. Therefore, large scale epidemiologic studies with sufficient numbers of blacks are need to determine if circulating 25(OH)D levels predict development of hypertension. This would help to establish the role of vitamin D as prophylaxis for hypertension and not just a treatment measure to reduce blood pressure.

In the CF community, translation studies to examine the impact of optimal vitamin D status on improving lung function would be beneficial. It is feasible that improving vitamin D status could have a much broader impact on the health beyond reduction in parathyroid hormone. Two such outcomes that would most benefit the CF community are lung function and reduction in respiratory illness. To study these two co-morbidities of CF, large clinical trials are needed. Since we were underpowered to

examine the effect of increasing 25(OH)D levels on lung function as measured by %FEV1, studies with larger numbers of participants would help to study this relationship. In addition, a large cohort study examining incident lung infections in individuals who had adequate vitamin D status would be beneficial. This would help to explain if any observed improvements in %FEV1 translate to reduction in the main co-morbidity associated with CF.

The second study in CF patients would be to examine the best repletion strategy for individuals who are acutely ill. We did not include any acutely ill individuals in the research presented here. If the gut is severely inflamed, it may not effectively absorb vitamin D_3, therefore UV light therapy may be the only option. For this study, I would recommend studying individuals with CF who have been admitted to the hospital. They would then be randomized to either UV light therapy or vitamin D_3 to determine which strategy best improved vitamin D status. This research would help determine if improving vitamin D status helps the acutely ill CF patient in addition to the relatively healthy patient. We could study duration of hospital stay and subsequent hospitalizations as possible outcomes.

Conclusion

Vitamin D deficiency is common and untreated the population at large and in particular in CF and hypertensive patients . Increased awareness of vitamin D deficiency and treatment options will help eradicate this disease. Additional studies exploring

mechanisms through which vitamin D reduces disease incidence and/or complications are needed to determine if there are disease specific treatment requirements. Treatment of vitamin D deficiency may benefit many individuals in addition to those with hypertension and CF.

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