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Vehicles for the Absorption of Vitamin D In Cystic Fibrosis: Comparison of Powder Vs. Oil

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ACCEPTANCE

This thesis, VEHICLES FOR THE ABSORPTION OF VITAMIN D IN CYSTIC FIBROSIS: COMPARISON OF POWDER VS.OIL, by Wendy A. Hermes was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT
VEHICLES FOR THE ABSORPTION OF VITAMIN D IN CYSTIC FIBROSIS: COMPARISON OF POWDER VS. OIL
By Wendy A. Hermes

Background Despite the high prevalence of vitamin D deficiency in cystic fibrosis (CF) populations, there is little consensus on the most efficacious vehicle substance for vitamin D supplements. Given the high prevalence of pancreatic insufficiency in CF, it is possible that resulting fat malabsorption may impede the ability of patients with CF to absorb vitamin D in an oil vehicle. Further investigation is needed to determine the optimal vehicle substance for use in vitamin D supplements. The objective of this pilot study was to compare the absorption of vitamin D_3 and to evaluate the rise in serum cholecalciferol ($D_{3)}$ concentrations in response to vitamin D supplements contained in power or oil vehicles. We hypothesized that vitamin D contained in a powder vehicle would be absorbed more efficiently than vitamin D contained in an oil vehicle in patients with CF.

Methods: This was a double blind, randomized control trial conducted in adult patients with CF during a hospitalization for at least 72 hours at Emory University Hospital for an acute CF event. This study was approved by Emory IRB. All subjects gave written informed consent for participation. Eligible subjects included adults with CF over the age of 18. Subjects were excluded on the basis of a history of hypercalciemia, chronic kidney disease (stage 3 or higher), FEV1% <20% or current hepatic dysfunction (total bilirubin > 2.5 mg/dL, direct bilirubin > 1.0 mg/dL). Subjects were randomized to either a one-

time bolus dose of 100,000 IU of vitamin D contained in a powder (BioTech Pharmacal Inc., 50,000IU/tablet, inactive ingredients gelatin: lactose, cellulose and magnesium stearate) or oil-based vehicle substance (Pro-Pharma, LLC ,10,000 IU/tablet, refined soybean oil and glycerin). Serum D_3 concentrations were analyzed at baseline, 12, 24, and 48 hours post-treatment and serum 25(OH)D_3 was measured at baseline, 12, and 24 hours. Group differences were assessed with repeated measures ANOVA. The area under the curve (AUC) for serum D_3 and the individual 12-hr time-point were also assessed as indicators of D_3 absorption in group comparisons (Student's t-test).

Results: This trial was completed by 16 subjects with CF. The mean age, BMI, and FEV_1% were 26.2±6.8 yrs., 20.4± 2.4 kg/m^2, 63±17%, respectively. The increase in serum vitamin D_3 concentrations was greater in the powder group ($p_{group*time}<0.001$). Serum vitamin D_3 was higher at 12-hours in the powder group compared to the oil group (144.32± 9.40 vs 80.0± 7.66 ng/dL, *p= 0.002*), although levels were similar between groups by 48 hours. Plasma 25(OH)D_3 concentrations increased significantly ($p<0.001$), although the effect was similar in both groups.

Conclusions: In adults with CF, a large dose of vitamin D_3 is more efficiently absorbed in a powder vehicle compared to oil by 12 hours after intake. Larger studies are needed to determine if the greater acute absorption of vitamin D_3 in a powder vehicle translates to better health outcomes compared to an oil vehicle. Funded in part by the Cystic Fibrosis Foundation.

VEHICLES FOR THE ABSORPTION OF VITAMIN D IN CYSTIC FIBROSIS: COMPARISON OF POWDER VS. OIL

by
Wendy A. Hermes

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ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
AAP	American Academy of Pediatrics
AI	Adequate Intake
AUC	Area Under the Curve
BMI	Body Mass Index
cm	centimeter
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFRD	Cystic Fibrosis Related Diabetes
D_2	Ergocalciferol
D_3	Cholecalciferol
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
$FEV_1\%$	Forced Expiratory Volume in one second

FFQ	Food Frequency Questionnaire
GA	Georgia
IOM	Institute of Medicine
IU	International Units
kg	kilogram
L	liter
LFFQ	Long Food Frequency Questionnaire
µg	microgram
mL	milliliter
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
nmoL	nanomole
oz	ounce
RDA	Recommended Dietary Allowance
SFFQ	Short Food Frequency Questionnaire
SNP	Single-Nucleotide Polymorphism
U.S.	United States of America
USDA	United States Department of Agriculture
UVB	Ultra-violet B

CHAPTER I

VEHICLES FOR THE ABSORPTION OF VITAMIN D IN CYSTIC FIBROSIS: COMPARISON OF POWDER VS. OIL

Introduction

Cystic fibrosis (CF) is a genetic disorder caused by a mutation in the long arm of chromosome 7 in the Cystic Fibrosis Transmembrane Conductance Regulator gene (CFTR)[1,2]. This gene mediates chloride ion secretion and regulates the transport of electrolytes across epithelial membranes. Mutations in this gene prevent the chloride channel from functioning properly, which impedes the normal flow of chloride ion and water into and out of cells. This results in abnormally thick and sticky mucus on the cells that line the lungs, pancreas, and other organs. This thickening of the mucus also increases the number of lung infections, which leads to damage in the airways and decreased lung function. The inflammation from these infections causes progressive damage to the epithelial lining of the lungs, which in turn shortens the life span of patients suffering from CF[3].

The mutated CFTR gene in the pancreas causes defective secretion of bicarbonate leading to pancreatic insufficiency and maldigestion of fat soluble vitamins and chronic nutrient deficiencies of vitamin A, E, D, and K. The Delta F508 mutation (a mutation causing the deletion of codons for phenylalanine, resulting in defective processing and premature breakdown of the CFTR) is the most common, occurring in approximately 80-

90% of patients. Infants are commonly diagnosed with CF shortly after birth with the first evidence of pancreatic insufficiency. The lack of digestive enzyme secretion is caused by inhibited exchange of Cl^- and K^+ ions from CFTR channel proteins, which ultimately inhibits endocrine pancreatic juice secretion, blocked ducts and fat malabsorption by the gut[4,5]. Enterically coated pancreatic enzymes are routinely taken at each meal by CF patients to enhance uptake of fat soluble vitamins as well to facilitate uptake of minerals that compete with fats for absorption.[1]

In 2011, The Cystic Fibrosis Foundation updated recommendations for vitamin D therapy in patients with CF, however, due to fat malabsorption problems in CF, there remains some question as to the most efficient vehicle compound for the vitamin D supplement for maximal absorption. A vehicle is a substance that may bind or carry the vitamin from one place to another and can include fats, water or acidic based substances. Vitamin D is a fat soluble vitamin and is more efficiently absorbed when combined with a fat/oils as the vehicle compound in healthy individuals and thus most vitamin D pills marketed in the U.S. are formulated with a fat soluble vehicle[6,7]. However, patients with CF are not able to efficiently absorb fats. Therefore, it is not known whether the vehicle substances of vitamin D supplements are poorly absorbed by CF patients. In this study we investigated the effectiveness of absorption of vitamin D in a powder bolus vehicle as compared to an oil based vehicle supplement in patients with CF. The hypothesis was that a powder based vitamin D_3 supplement yields a higher rate of absorption than an oil based supplement.

CHAPTER II

REVIEW OF THE LITERATURE

Functions of Vitamin D

The role of vitamin D_2/D_3 in bone health is well documented. Vitamin D_2, ergosterol, comes from plant sources, whereas vitamin D_3, cholecalciferol, is either produced in the skin upon contact with UVB radiation and ingestion of oil-rich fish[8,9]. Both forms are available from fortified dairy and cereals. Vitamin D_2 and D_3 must first be hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D), and then in the kidneys to form 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), to become an active hormone. Activation to the biological form in the kidneys requires a hydroxylase enzyme (25(OH)D-1-OHase)[8]. The hormonal form of vitamin D, $1,25(OH)_2D$, travels to tissues with a vitamin D binding protein (DBP)[8]. Once it reaches a target tissue, $1,25(OH)_2D$ interacts with vitamin D receptors (VDRs). Such receptors are found in multiple tissues throughout the body such as small intestines, bones and kidneys[9]. Vitamin D is thus able to serve several well established functions, in addition to its most well-defined roles in calcium homeostasis and bone maintenance. New studies present evidence that vitamin D plays a role in immunity, genetic transcription and lung health[9,10,10–12]. Often studies involving vitamin D and immune health have focused on doses higher than the recommended intakes for healthy populations[13–15].

The Institute of Medicine has currently set recommended daily intakes (RDI) for vitamin D at 600 IU/day in ages 1-70 and 800 IU/day for >70 years, not to exceed 4000 IU as the Upper Limit (UL)[16]. In the last 10 years several studies have explored doses exceeding the UL in relation to vitamin D in its relation to hormonal function and immune health[6,13,17]. Outcomes used to assess this relationship include FEV 1, LL-37/cathelicidin[13,14,18]. A 2011 review on vitamin D and lung health cited studies relating higher doses of vitamin D with enhanced FEV1 using cross-sectional NHANES III data in non-CF populations[9,19]. FEV1 is also used as a measure of respiratory function related to pathogen growth in the patients with CF, tuberculosis, asthma, and other respiratory diseases[14,20,21]. Studies have illustrated the role of vitamin D in the induction of the antimicrobial peptide, cathelicidin (LL-37), exhibiting the body's initial defense against airway pathogens such as Gram-positive, Gram-negative bacteria, fungi, and viruses[18]. Active $1,25(OH)_2D$ is necessary to bind to the nuclear receptor, VDR, to increase mRNA expression of cathelicidin in both healthy and compromised lungs.[9,18] Associations between vitamin D status to the positive genetic expression of at least 160 pathways which ultimately may affect outcomes in autoimmune disorders, cancers, and cardiovascular disease have been promising[22]. A randomized clinical trial by Hossein et al in 2013 was important in defining functions of vitamin D_3 that have been previously elusive, making the deficiency in this vitamin a prime concern for certain populations affected by genetic immunity disorders

Vitamin D Deficiency in Non-CF Populations

In 2011 The Endocrine Society updated guidelines for vitamin D supplements and treatments in the general population[8]. These updates occurred in light of vitamin D deficiencies within the general population, indicating an increased public health risk.[1,8] An NHANES analysis for the years 1988–1994 and 2000–2006, examined hypovitaminosis D within the general population while adjusting for updated assay drifts over time, reiterating a general vitamin deficiency while validating assay testing. This study emphasizes that higher prevalence of hypovitaminosis D in 2001–2006 compared to 1988–1994, with an increase of 5-10% in all people with serum 25(OH)D <30 ng/ml (3 to 8% in men, from 22 to 38% in blacks, from 3 to 8% in 12- to 15-y-old adolescents, from 5 to 12% in 20- to 30-y-old adults, from 6 to 14% in non-supplement users, and from 8 to 17% in persons with BMI >80th percentile).[19] The established deficiency rate for all populations was previously described by Holick et al. in 2011 as 25(OH)D < 20 ng/ml (50 nmol/liter), and insufficient as 25(OH)D of 21–29 ng/ml (525–725 nmol/liter).[8] In response to a vitamin D deficiency within the general population, in the 2012, the CF Foundation also updated its guidelines for vitamin D supplements and treatments in populations diagnosed with CF.[1]

Prevalence of Deficiency in CF Populations

The prevalence of vitamin D deficiency in CF has been well described from birth through adulthood[1]. Vitamin D status begins to decline during adolescence and some study centers report insufficiency between 81 and 90% by adulthood[15,23,24]. Decreased

bone mineral density in adulthood may be described by a high frequency of early diagnosis of vitamin D deficiency in CF youth[25].

Production of vitamin D in the skin upon exposure to sunlight is one of the primary sources of vitamin D in the human body. While the majority of healthy populations get 95% of vitamin D from sunlight, exposure to sunlight is reduced in CF. Individuals with CF have frequent infections requiring hospitalization and are often prescribed antibiotics causing increased photosensitivity which further limits exposure to sunlight[26]. Antibiotic regimens containing chlorines or floroquinolones commonly cause UV induced skin rashes, thus making the use of sunscreens a necessity while reducing vitamin D producing capacity in the skin, [27–29]. Therefore, skin formation of vitamin D in individuals with CF is reduced compared to individuals without CF and may be one of the primary reason for decreased vitamin D status[1,30]. Sun exposure is further reduced in higher latitudes (47 degrees latitude) posing additional risk for deficiency due to decreased UVB penetrating the earth's surface for cutaneous vitamin D production[31].

Malabsorption of fat soluble vitamins due to pancreatic insufficiency is perhaps the most significant barrier for nutrient absorption in CF. Pancreatic scarring and fibrosis causes blockage of digestive enzyme secretion, limiting the absorption of fat soluble vitamins[2]. Even in the presence of enzymes, research has revealed a reduced level of vitamin D absorption in CF. Lark et al. demonstrated that patients with CF only absorbed approximately half of the amount of vitamin D administered compared to healthy controls[32].

Although malabsorption and low sun exposure are primary contributors to vitamin D deficiency in CF, more than 20 studies highlight other possible reasons for low vitamin D metabolism[29]. Inadequate intake of vitamin D fortified foods and supplements, in addition to pancreatic exocrine function, limits vitamin D bioavailability[25,33]. Impaired hepatic hydroxylation, accelerated excretion of vitamin D and decreased expression of vitamin D binding protein (VDP) may also contribute to deficiencies[29,32,34].

Implications of Vitamin D Deficiency in CF

The role of vitamin D in skeletal health in the general population and CF is well established. Several CF centers have established vitamin D deficiency as a risk factor for low bone mineral density in patients with CF and cite preventative supplementation[11,35–38]. However, despite the strong association linking vitamin D and skeletal health, there have been few random-controlled trials (RCT) proving that low BMD is a direct outcome of a vitamin D deficiency[39]. A 2010 Cochrane Review outlined only 2 RCTs (Popescu data only,1998 and Haworth, 2004) illustrating a BMD outcome after a vitamin D supplement with placebo[39–41]. Due to its inextricable role in calcium and parathyroid hormone homeostasis and (bone remodeling), it is difficult to either exclude or definitely prove vitamin D's true magnitude in bone density. The current association between CF, vitamin D, and skeletal health has primarily been studied through retrospective and cross-sectional studies assessing BMD directly while also looking at vitamin D deficiencies in the samples[24,42]. For example, half of the subjects in Elkin et al. had a mean serum 25(OH)D concentration of 11 ng/mL and 36% of subjects having a serum 25(OH)D < 10 ng/mL. From this population, 27% also had at least one vertebral fracture and 11% had two vertebral fractures over a 2-year period[42]. This study demonstrated an association

between low serum 25(OH)D and the increased risk for fracture, but does not clearly prove the cause of these bone density related fractures.

Lung function and the ability to fight infection are both important in the CF population as a predictor of morbidity and mortality[14,43]. Decreased serum 25(OH)D is associated with decreased lung function as measured by Forced Expiratory Volume in one second (FEV1), as well as a higher frequency of bacterial colonization from bacteria such as P. aeruginosa[44]. Low vitamin D status is associated with an increased number of hospital stays and ultimately increased mortality in CF[1,14,29]. One cross-sectional study examined incidence of the first P. aeruginosa infection and percent predicted lung function in children aged 8, 12, and 16 years. The study concluded that higher serum 25(OH)D levels in children with CF were associated with fewer pulmonary exacerbations, and in adolescents, higher FEV1[43]. This result supported conclusions previously demonstrating an association between vitamin D status and lung function in non-CF populations[20].

Vitamin D status may also play a role in CFRD. Diabetes develops in up to 25% of young adults, and up to 50% in later adulthood[45]. Research in the non-CF population suggests that vitamin D may have a positive effect on insulin sensitivity in that it may help lower serum glucose and mediate insulin sensitivity[46–49]. However, more random controlled trials are needed in the CF population to more clearly define this role. One RCT in CF, Pincikova et al., studied CF patients from Sweden, Norway and Denmark and demonstrated a positive association between glycosylated hemoglobin (HbA1C) and vitamin D insufficiency suggesting a link between vitamin D deficiency and pancreatic endocrine insufficiency in CF[50]. Pincikova et al suggests that vitamin D supplementation

improves insulin secretion and sensitivity by reducing inflammation. Inflammatory markers such as C-reactive protein, IL-1, IL-6 and TNF-alpha are elevated in CFRD as well as insulin resistance, type 2 diabetes[46].

Vitamin D and inflammation are strongly associated with CF infections. A 2011 study of CF respiratory epithelial cell lines exposed to Pseudomonas aeruginosa found a decrease in inflammatory cytokines IL-6 and IL-8 when exposed to the hormonal form of vitamin D, 1,25(OH)2D[51]. Vitamin D may up-regulate anti-microbial peptides, namely cathelicidin, to enhance clearance of bacteria at various barrier sites and in immune cells[52,53]. A 2009 review examined literature regarding clinical evidence for vitamin D as a modulator of the innate and adaptive immune system[53]. Cathelicidin exhibits a bacterial killing response in humans and is expressed by epithelial cells of respiratory tract. In people with reduced cathelicidin, bacteria accumulation increases inflammatory responses[53]. A randomized placebo-controlled trial in adults with CF comparing vitamin D vs placebo found a statistically significant 50.4% reduction in tumor necrosis factor-α (TNF-α) and a trend for a reduction in IL-6 in CF patients given a bolus dose of 250,000 IU of vitamin D_3[14]. Therefore, vitamin D may have multiple effects on innate immunity by increasing anti-microbial peptides and by mediating inflammation.

While there has yet to be a definitive recommendation for vitamin D formulation, there have been studies using both vitamin D_2 and D_3 [13–15,17]. There have been mixed results on the effective absorption of vitamin D_2 in CF populations, however, vehicles used in clinical studies were primarily oil based (comprised of refined soybean oil and glycerin)[1,8,32,54,55]. Conversely, clinical trials supplementing vitamin D_3 have mainly

employed the use of a powder vehicle (comprised of lactose, cellulose and magnesium stearate)[6]. The Endocrine and Cystic Fibrosis Society now recommend the use of vitamin D_3 for supplement use[1,8]. This recommendation is based on results indicating that cholecalciferol (D3) may exhibit greater pharmacokinetic potency than ergocalciferol (D_2), and as such will be better absorbed in CF than D_2. Green et al 2008 and Rovner et al 2007 (respectively) both describe high dose vitamin D studies in children and adults with CF, finding that vitamin D_2 did not maintain serum 25(OH)D at sufficient levels[25,56]. Conversely, a single randomized trial directly compared side by side an oil vehicle of D_2, a lactose based vehicle of D_3, and cholecalciferol production by UVB in CF patients. While this trial found both D_2 and D_3 adequate in raising serum 25(OH)D to sufficient levels (>30 ng/mL), the results were confounded by the difference in the vehicle of absorption[54]. Only one study compared vitamin D_2 and D_3 side by side in healthy populations with 25,000 IU of vitamin D in whole milk compared to the same dose in oil on toast[57]. The authors then performed a study to demonstrate that vitamin D fortified orange juice (D_3) could be used to successfully raise vitamin D status in healthy individuals[17]. We propose that the vehicle may be the reason for differences in absorption of vitamin D_2 and D_3 in CF patients. Given that fat contents are malabsorbed in CF, we hypothesized that an oil form of vitamin D_3 will be less effectively absorbed than a powder bolus vehicle in CF patients.

This proposal hopes to close a gap in vitamin D guidelines for the CF population. The CF Foundation has been unable to update recommendations on the vehicle (i.e. substance compound of the vitamin D supplement) for vitamin D supplements due to insufficient studies[1]. A comprehensive 2010 review of vitamin D vehicle trials identified

and compared four studies for this purpose, comparing oil, cellulose/lactose, and ethanol as absorption mechanisms, in healthy subjects[6]. In this literature review concerning non-CF subjects, oil was concluded as the most efficacious vehicle, however the studies using oil were only partially verified and had confounding variables[6,58–61]. There have been no clinical trials conducted in the CF population to evaluate the impact of the vehicle substance on absorption of vitamin D. This lack of evidence indicates a need for clear delineation in the CF population for either a oil or powder vehicle.

The objective of this study is to combine current resulting factors of digestion, absorption and pharmacokinetics of vitamin D in determining the most effective vehicle for CF patients. **Based on the current understanding that fat is malabsorbed in CF, the hypothesis is that a powder mechanism will support better overall absorption in this population.** Determination of such a pathway would be valuable in the future treatment not only in CF, but of other vulnerable populations with increased risk for malabsorption disorders[6].

CHAPTER III

METHODS AND PROCEDURES

We proposed a randomized double-blind clinical trial of approximately 12-24 CF subjects (>18 yrs. old) to be recruited at Emory (Emory University Hospital) to compare the absorptive efficacy of an oral powder vehicle or oil vehicle containing a total of vitamin D_3 (100,000 IU) supplement. Baseline studies were conducted upon hospital admission and informed consent. Urine was collected prior to enrollment to perform a pregnancy test in all females of child-bearing potential if was not already been performed as part of standard of care procedures. During a 3-4 day hospitalization, subjects were randomly assigned to one of two groups. Group 1 received a powder vitamin D_3 bolus and Group 2 received an oil supplement of 100,000 IU vitamin D_3 each. Information was extracted from the medical record and from questions to the participant to assess current vitamin supplementations, enzyme use and current overall health concerning CF. Serial samples for blood markers for vitamin D_3 status (serum vitamin D_3 and plasma25(OH)D).

Clinical data were abstracted from electronic medical health records. Clinical data collected included age, gender, ethnicity, BMI, genotype, co-morbidities such as kidney or liver disease, antibiotic use, HbA1C, most recent plasma 25 hydroxyvitamin D_3 [25(OH)D], and FEV% in 1 second as a measure of lung function. Questionnaires for research purposes were given to participants after enrollment to collect data for recent

sun exposure, smoking status/packs per day, alcohol consumption, current vitamin D supplementation and enzyme use.

Recruitment was performed through referral from the Emory University Hospital service caring for CF patients and the outpatient CF Clinics. At Emory, referrals were received from the Emory University Hospital Clinic. Screening of new inpatients was completed daily by a research coordinator. Patients who met the following inclusion criteria and met none of the exclusion criteria on the initial assessment were offered the opportunity to participate in the study. The study coordinator explained the study protocol in detail in the consent form as well as verbally to prospective participants.

Intervention

Patients were randomly assigned to a powder supplement or to an oil based supplement each of 100,000 IU vitamin D_3. Patients were randomized following recruitment in blocks of 4 (meaning for every 4 subjects there will be 2 powder treated patients and 2 oil treated patients). Blood was drawn by IV catheter at baseline, 2, 4, 8, 12, 24, 48, and 72 hours after vitamin D_3 dosing. The powder bolus, Vitamin D3-50 Cholecalciferol, was obtained from BioTech Pharmacal Inc. (50,000IU/tablet, inactive ingredients gelatin: lactose, cellulose and magnesium stearate). The cholecalciferol oil-based supplements were obtained from Pro-Pharma, LLC (Maximum D3, 10,000IU/tablet, refined soybean oil and glycerin). A Certificate of Analysis was obtained from Analytical Research Laboratories (ARL; 840 RESEARCH PARKWAY, SUITE 546 OKLAHOMA CITY, OK 73104) to validate capsule content potency of both the powder and the oil D_3 capsules. The 50,000IU powder capsule was found to contain

91.3% of the stated amount of vitamin D and the oil capsule was found to contain 95% of the stated amount of vitamin D.

Follow-up

Patients received a validated 3-day food diary to complete after release from the hospital and to return at a later date. The food diary was used to assess a habitual intake of vitamin D per subject to account for variations of vitamin D_3 found in blood due to intake. Follow-up phone calls were made to ensure compliance for the food diary. Questionnaires were used as validation measures for relating blood levels of vitamin D_3 after dosing.

Inclusion Criteria

Adult CF patients (>18 yrs), Able to tolerate oral medication, Expected to survive the duration of the study.

Exclusion Criteria

Inability to obtain or declined informed consent from the subject and/or legally authorized representative; a history of disorders associated with hypercalcemia and/or current hypercalcemia (albumin-corrected, serum calcium >10.8 mg/dL or ionized calcium >5.2 mg/dL), Chronic kidney disease worse than stage III (<60 ml/min), FEV1% predicted <20%, Current significant hepatic dysfunction total bilirubin > 2.5 mg/dL with direct bilirubin > 1.0 mg/dL, Current use of cytotoxic or immunosuppressive drugs; History of AIDS or illicit drug abuse; too ill to participate in study based on investigator's or study team's opinion; current enrollment in another intervention trial.

Biochemical Analysis

Serum cholecalciferol at times 0, 12, 24, 48, and 72 was measured with LC-MS/MS at Heartland Assays, Ames, IA. Peak absorption of cholecalciferol was assessed using the 12-hr time point, based on two preceding published recommendations by Lo et al and Farraye et al[62,63]. The area under the curve (AUC) for serum cholecalciferol over all time points, calculated using the trapezoidal method[64], was also assessed as an indicator of absorption. A higher 12 hour serum cholecalciferol or higher AUC reflects greater cholecalciferol absorption. Plasma 25(OH)D and PTH were measured with automated chemiluminescence assay (iSYS System, Immunodiagnostic Systems Inc., Gaithersurg, MD, USA). External quality assessment is monitored annually through the vitamin D external quality assessment scheme (DEQAS) certification. This measure ensures that 75% or more of the 'useable' results (generally 16) should fall within ± 25% of the Target Value[65]. To minimize interassay variability, all samples were analyzed simultaneously.

Statistical Analysis and Power

Descriptive statistics were performed and reported as mean ± SD or n (%). Vehicle (oil vs powder) group differences in baseline demographics were analyzed with Fischer's Exact tests for categorical variables and student's t-tests for continuous variables. Student's t-tests were used to examine vehicle group differences in the AUC for serum cholecalciferol over 48 hours and at the individual 12-hour time point. Two-way mixed model repeated measures ANOVA was used to test for differences in serum cholecalciferol and 25(OH)D by group, over time, and in the group by time interactions.

Post-hoc treatment and individual time responses were analyzed using Tukey's T-tests ($p<0.05$). Statistical analyses were performed with JMP Pro ™ 10.0.0 (SAS institute Inc., Cary, NC) using two-sided tests and assuming a 5% significance level. As this is a novel pilot study and there are no previously published trials from which to draw power calculations, a formal power analysis was not conducted. We aimed to recruit 12-24 subjects to establish feasibility and produce needed preliminary data for a future, adequately powered, robust clinical trial.

CHAPTER IV

RESULTS

Subject Demographics

A CONSORT diagram of study enrollment is provided in Figure 2. A total of 17 participants were randomized to receive 100,000 IUs of cholecalciferol in either oil or a powder form (Figure 1). Results of the study are reported only for participants who completed at least 0, 12, and 24 hour time points (n=6 in powder group, n=9 in oil group). One participant withdrew from the study prior to completing all crucial time points and was therefore excluded from final analysis. A second participant was excluded from final analysis for an inability to acquire an adequate blood sample at the 12-hr time point.

Baseline demographic and clinical characteristics are presented in Table 1. The demographics for each group are approximately evenly distributed with no significant differences between oil or powder as the cholecalciferol vehicle. The mean age of the group was 26.2±6.8 years, approximately 50% were female and 88% were of Caucasian ethnicity. One participant was of African American decent and one of East Asian descent. The mean BMI was approximately 20 kg/m^2. Only two participants were not on a pancreatic enzyme regimen, and 29% were currently prescribed a vitamin D supplement. CF related diabetes status and insulin resistance was noted from patient medical charts. Patients were rated as having normal blood glucose, impaired fasting glucose, impaired glucose tolerance, CFRD with fasting hyperglycemia, CFRD without fasting hyperglycemia, or unknown. A diagnosis of CFRD and insulin resistance was obtained on the basis of A_1C% (n=13) and oral glucose tolerance testing (2). Four subjects met one of the above criteria; two had impaired fasting glucose, one had impaired glucose

tolerance, three had CFRD with fasting hyperglycemia and three were of unknown status as of their last physical exam. Most participants (75%, n=12) had recent serum 25-hydroxyvitamin D levels below CF sufficiency recommendations (30 ng/mL); three (25%) in the insufficient (20-30 ng/mL) and eight (66%) in the deficiency ranges (<20 ng/mL). The mean FEV % in 1 second was 49.1%

Changes in Serum Cholecalciferol Over time

Serum Cholecalciferol measurements for the powder group at baseline, 12, 24, and 48 hours were 4.62 ± 9.40. As shown in Table 2 (Figure 3), there was a statistically significant time effect (repeated measures ANOVA P_{time} <0.0001) indicating that serum cholecalciferol increased over 48hrs in both the oil and the powder groups. The group-by-time interaction was statistically significant ($P_{group*time}$<0.0001), reflecting a higher serum cholecalciferol response in the powder group (Figure 2). Serum cholecalciferol was higher at 12-hrs in the powder group compared to the oil group (144.32± 9.40 vs 80.0± 7.66 ng/dL, P = 0.002). The powder group increased by an average of 140 ng/ from baseline to a peak at the 12 hour time point, whereas the oil group increased an average 74 ng/ml by 12 hours. The D_3 in the oil group following the 12 hour time point continued to climb another 15% to reach its peak at 93.7 ng/dL before descending back to 60 ng/dL by 48 hours *(p<.05*). The powder group had a mean decrease of 74% from the 12 to 24 hour point (37 ng/dL) and an additional 32% from 24 to 48 hrs (50 ng/dL). All changes between time points 12 to 48 were statistically significant (p<.05) with the exception of points between 12 and 24 hours in the oil group. Powder and oil serum cholecalciferol were equal by the 48 hour time pint with 60.2 and 60.0 ng/dL respectively. The AUC for

serum cholecalciferol (Figure 4) was also higher in the powder group compared to the oil group (3256.5±285.5 vs 2393.73±233.1, p = 0.036).

Plasma 25-hydroxyvitamin D by time and group

Two-way repeated measures ANOVA analyses (Table 3, Figure 5) indicated that serum 25-hydroxyvitamin D (25(OH)D) increased significantly by time ($p_{time}=0.006$), although the group effect and the group by time interaction were not different ($p_{group} = 0.347$, $p_{group*time} = 0.300$). Serum 25-hydroxyvitamin D increased from levels considered deficient (<20 ng/mL) meet the recommended range for vitamin D sufficiency at (>30 ng/mL) within 24 hours in the powder group.

CHAPTER V

DISCUSSION

This pilot study examined differences of absorption of two opposing vehicles of a vitamin D3 supplement in people with Cystic Fibrosis. Cystic Fibrosis is a genetic disorder affecting the exocrine function of the pancreas through scarring and limited release of digestive enzymes into the small intestine. Pancreatic enzymes and bile are the primary mechanism for the absorption of fats and fat soluble vitamins; thus in CF, these are markedly decreased. In non-CF groups dietary intake is absorbed most efficiently in foods containing fats. A 2014 randomized control trial compared plasma D_3 in healthy adults after a 30% fat meal compared to a fat-free meal, finding that subjects consuming the fat containing meal absorbed 32% more of a 50,000 IU vitamin D supplement than the fat free group ($P=0.003$)[7].

Pancreatic scarring in CF decreases available enzymes that aid absorption. Although pancreatic enzymes replace this deficit, people with CF still exhibit malabsorption compared to their non-CF counterparts when consuming vitamin D with oils[54]. We proposed that a supplement prepared in a water soluble powder vehicle (powder composed of lactose, cellulose and magnesium stearate) would be more freely absorbed than an oil preparation (refined soybean oil and glycerin) in CF since absorption of water soluble vitamins does not require micellar packaging or lipase enzymes. Other

common CF fat soluble vitamins already are routinely prepared within a water soluble format such as lactose, cellulose and/or magnesium stearate[66].

This study found a significant rise in serum cholecalciferol from a powder compared to an oil vehicle after a bolus dose of 100,000 IU vitamin D_3 in CF patients ($p<0.05$). At baseline, 11 study subjects exhibited cholecalciferol levels of five ng/dL or less. Four subjects had levels less than 10 ng/dL, and one subject had a level of 22.5 ng/dL. We found the peak absorption in powder to occur at approximately 12 hours and in oil to be approximately 24 hours. This is consistent with two studies that both examined vitamin D absorption in people with intestinal malabsorption compared to healthy controls. Lo et al. 1985 developed an oral challenge test to quantifying the difference in absorption between healthy controls and patients with fat malabsorption syndromes using a 50,000 IU bolus dose of D_2, and found that serum cholecalciferol (ng/dL) began to rise four hours after ingestion and 25- hydroxyvitamin D began to rise 12 hours after ingestion.[63] Farraye et al. 2011 further demonstrated a 12 hour peak by vitamin D supplementation in patients with Crohn's disease. Additionally, this clinical trial was able to show that anatomical location of malabsorption did not affect absorption of vitamin D[62]. The present study is consistent with this data insofar as cholecalciferol at baseline was 7 ng/dL or less for all subjects and was significantly increased by 12 hrs. in powder. We found no significant difference between subject groups at baseline.

Following peak absorption, both the powder and the oil group had similar vitamin D_3 levels by 48 hrs. post dose. There was a similar significant rise of serum 25(OH)D in both groups from baseline and the powder group had a higher trend toward the sufficiency range than the oil group at peak (29, 22.9 ng/mL respectively); however, the

difference between the groups was not significant (p=0.347). Previous research hypotheses indicate that powder may be more bioavailable in patients with malabsorptive disorders[6,54]. This is the first trial to do a direct comparison of D_3 with powder and oil in CF. Khazai et al. 2009 found that 25(OH)D increased significantly via a powder bolus of D3 compared to D2 in oil[54]. While this study was longer in duration than the present study and also compared different vitamin D compounds (D_2 vs D_3), the trend of our results are consistent with this hypothesis/result. Since this publication, the CF Foundation has advocated for the use of D_3 in CF based on its confirmed effectiveness in raising 25-hydroxyvitamin D over Vitamin D_2[1]. The results in the vitamin D metabolite, 25(OH)D, do not show a similar parallel rise in the powder group. Since it is hypothesized that conversion of cholecalciferol to 25(OH)D is dependent on circulating levels of cholecalciferol, we expected a more significant hepatic effect in the powder group[62].

Serum cholecalciferol represents the direct absorption of vitamin D from the digestive track and excludes that of the skin. Our result indicated that cholecalciferol appropriately measures absorption, yet its overall effectiveness in promoting vitamin D status in CF is still unclear. One possible reason for this difference may be the time factor in the pilot design of our trial. Khazai et al. 2009 examined 50,000 IU over 12 weeks as compared to a one time bolus dose of 100,000 IU evaluated over a 48 hour period. It is possible that while this dose was enough to show absorption differences in D3, it was still not enough to compare a difference in total vitamin D status. Conversely, the Khazai trial found differences that may have been solely related to the vitamin D compound (D_2 vs D_3). Based on our data alone, it is difficult to extrapolate the reason for the difference in

rate of hydroxylation of the powder form of D_3 to 25(OH)D compared to the oil, whether the difference is related to factors associated with CF. Although this trial exhibited that a cellulose powder was more effectively absorbed into blood, clinical practice guidelines indicate total 25-hydroxyvitamin D to be a better indicator for overall vitamin D status and a clinical marker of health[23,67,8]. Additional studies utilizing longer follow-up periods may reflect how absorption of cholecalciferol may impact the pathway of hepatic 25(OH)D.

There are several hypothesis to describe possible reasons for reduced levels of 25(OH)D in the CF population. After ingestion, cholecalciferol is hydroxylated in the liver by hepatic 25-alpha hydroxylase enzyme. There is still limited evidence detailing the genetic expression of this hepatic enzyme. Zhu et al 2013 has isolated CYP2R1 and a second unknown enzyme as the genetic SNP (single-nucleotide polymorphism) for hepatic 25-alpha hydroxylase by using double-knockout mice[68]. Additionally, Engelman et al 2013 also found that a higher dietary exposure of vitamin D was strongly related to SNP expression of CYP2R1, suggesting a possible nutrigenomic effect on enzyme expression and thus potential 25(OH)D levels[69]. A second hypothesis describing low 25(OH)D levels is that sequestration of vitamin D in lipid cells. Due to its fat soluble nature vitamin D may be stored in fat cells within either visceral, subcutaneous or intramuscular lipid cells, however, research has been inconclusive due to the variability of study designs on subjects employed[70–72]. People with CF often have low body weight independent of body fat, however, since body fatness is not typically measured as part of the CF standard of care, we cannot yet hypothesize how vitamin D status is affected by

body fat in CF. Additional studies evaluating the relationship between lipid storage and muscle mass in both the general population and CF are needed.

Strengths and Limitations

This clinical trial had several limitations. The study was limited in its ability to assess long-term vitamin D status in a CF population since samples were collected only up to the first 72 hours after dosing. Collection points for 25(OH)D after 24 hours were reduced in power due to subject hospital discharge. Therefore, while we did find a significant difference in absorption between the two vehicles in the serum cholecalciferol, questions related the secondary clinical outcome with the use of a powder vehicle dosing are still unclear. Our primary objective in this pilot study, however, was to measure short-term absorption to determine vehicle efficacy.

The small sample size prevented covariate analysis of any baseline variables to the primary study outcome. Baseline characteristic were uniformly distributed between groups at enrollment and randomization, however, there were two male subjects excluded from final analysis due to inadequate numbers of sample obtained during the intervention. The final analysis for each group was n=6 powder and n=9 oil and 62.5 and 33.3% female respectively. Although this gender difference is not statistically significant, the sample size prevents an accurate representation of a more robustly powdered trial. Gender is not a determinant in absorption in healthy subjects[73] and it has not previously been indicated that gender is a determinant of nutrient absorption in CF[1]. Furthermore, there are no gender differences in the prevalence or incidence of CF currently known.

Larger trials that examine gender differences in absorption of vitamin D in powder are required to determine if this association exists in the general CF population.

Finally, the sample in the present study represents a small portion of the CF population; recruited as a consequence of their admittance to EUH for an acute CF event. At the time of enrollment, the overall health status of study subjects was considered "poor health", and thus, may not be representative of the absorption occurring in a healthy person with CF. Although Cystic Fibrosis can be considered a disease of chronic inflammation, it is unknown the extent to which CF acute illness has on metabolic processes such as nutrient absorption. Trials that compare healthy people with CF and those with an acute lung infection will further explain this difference.

Strengths of this study are the randomized control design. Furthermore, this is the first trial to measure a direct comparison on two vehicles of cholecalciferol in an adult CF population. The CF population is approximately 30,000 individuals with 1/3200 new diagnoses each year. This is a relatively small population and as such the sample size of the present study was both adequate and feasible given available resources for recruitment and time. Furthermore, a significant effect was achieved in the powder group which had three fewer subjects on final analysis than the opposing oil group. A larger trial of similar design and structure would most likely magnify this result.

Clinical Significance and Conclusions

In this randomized pilot trial we provided a 100,000 IU bolus dose of vitamin D_3 to 17 subjects with Cystic Fibrosis in either a powder or an oil vehicle to evaluate absorption efficacy. This is the first clinical trial to evaluate such a direct vehicle

mechanism of vitamin D_3 absorption. Although the powder mechanism was found to significantly increase D_3 absorption, reaching a peak at 12 hour post ingestion, both the powder and the oil groups had a similar rise in the clinical metabolite of vitamin D (25(OH)D). We found no baseline differences in nutritional status nor habitual intake of vitamin D foods to be an influence on either absorption or hydroxylase activity in this particular trial, however, a closer look at variables describing body composition and nutrigenetic effects of foods in a CF population would give a more detailed picture of metabolic pathways in vitamin D metabolism and overall health status.

We observed a significant rise in 25(OH)D from baseline in both groups, indicating the overall effectiveness of D3 in bringing patients with CF in sufficiency range. However, extended follow-up studies are required to evaluate long term health effects.

Some of the above questions may be addressed in the future by attempting a closer evaluation of in vitro muscular lipid metabolism in CF, taking into account the possible effects of CFTR mutations on transcription of the hepatic hydroxylation enzyme, 25-alphahydroxylase. This study did not collect the specific CFTR mutation type for each subject, yet several new mutation specific treatments are currently in use in pulmonary therapies. Research assessing muscular metabolism in CF could potentially benefit patients that meet adequate nutritional status through BMI yet remain at risk for low bone density due to consistent vitamin D inadequacy. It has also opened additional areas of possible clinical research that may add to the limited body of evidence available to CF clinicians.

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APPENDICES

Figure 1 Intervention timeline Patients with Cystic Fibrosis admitted for acute illness will be screened, randomized into two groups and monitored via blood samples for up to 72 hours.

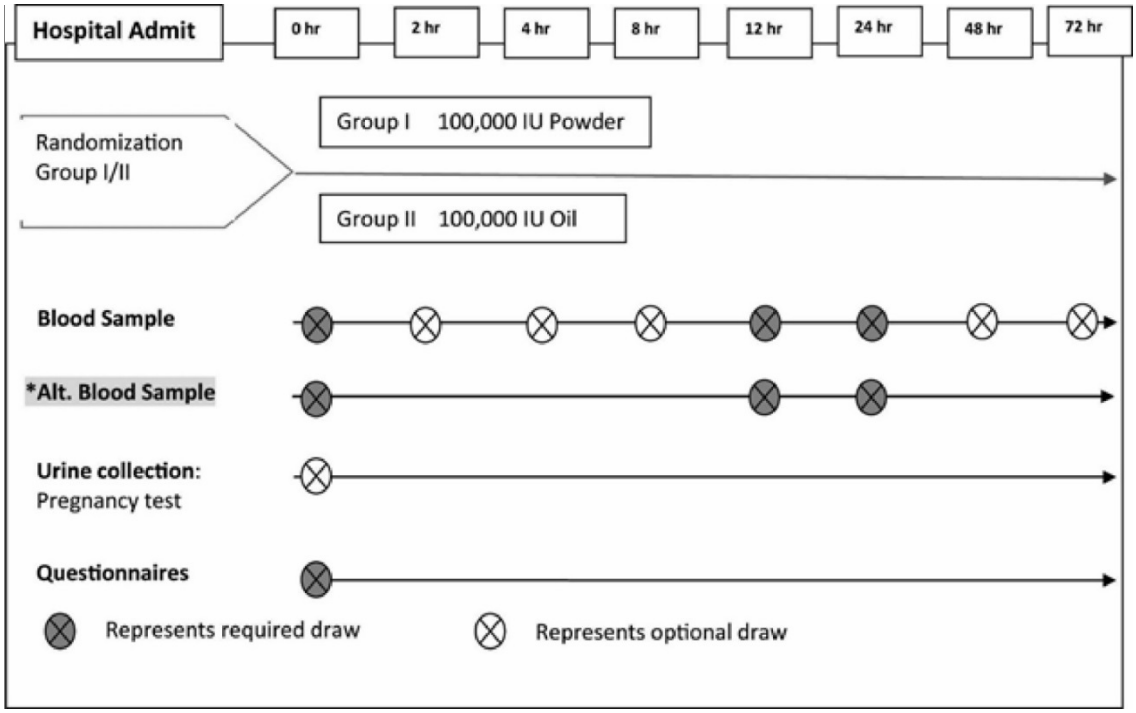

Figure 2. Flow diagram of participant enrollment. Subjects with Cystic Fibrosis (n =37) were screened for participation in the study. Seventeen subjects were enrolled and randomly assigned to receive either a powder or an oil vehicle of vitamin D_3. One subject did not complete the intervention and one subject was excluded due to inadequate sample collection.

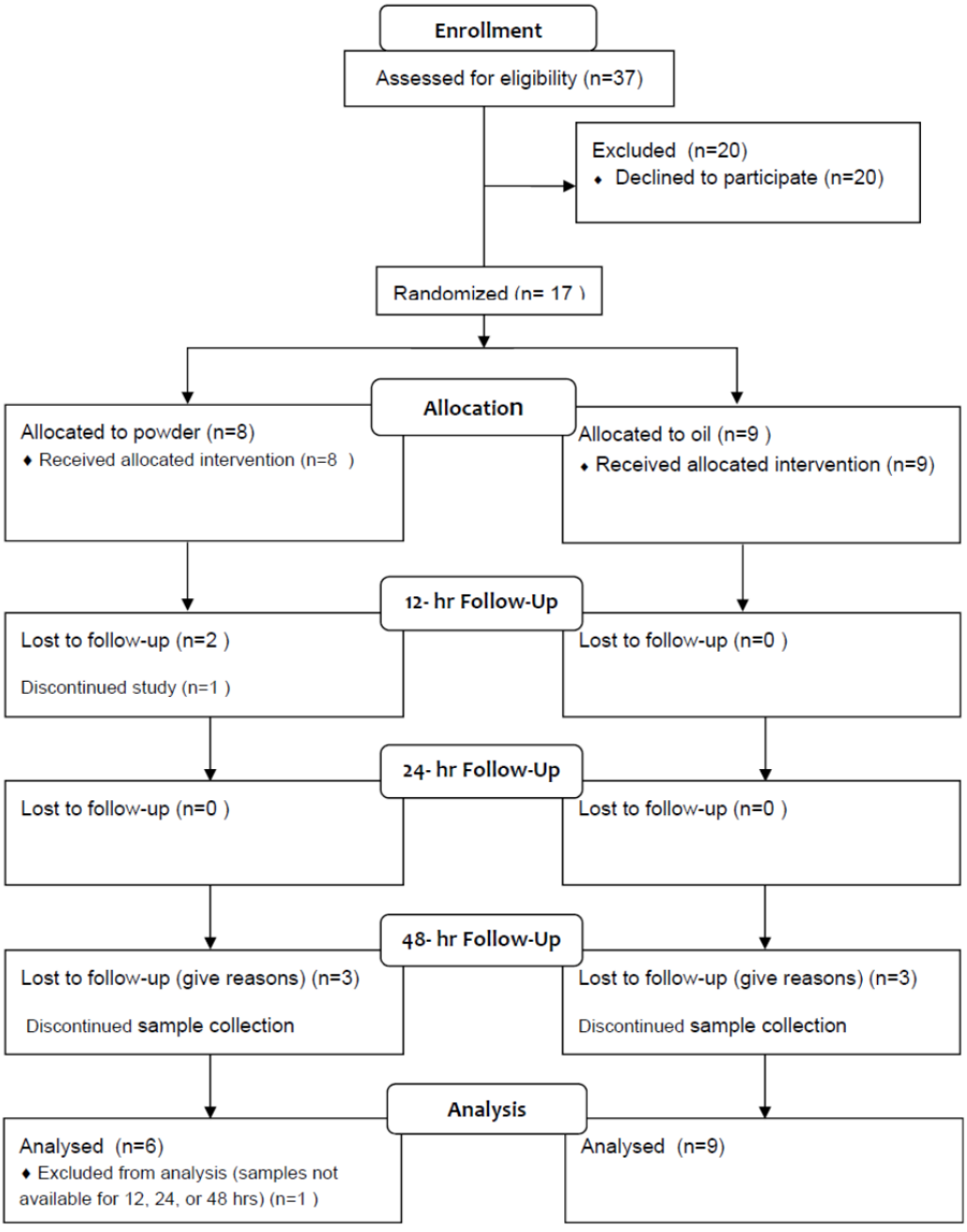

Table 1. Participant Demographics

DEMOGRAPHIC	Powder (n=8, ±SE)	Oil (n=9, ±SE)	p-value*
Age (yrs)	27.7 ± 2.3	25.7 ±2.0	0.77*
Gender (%female)	62.5	33.3	0.35**
Ethnicity (% white)	71.0	100.0	
Smoking	0	0	1.0
BMI (kg/m2)+	20.3 ± 1.1	21.1 ± 1.0	0.62*
Enzymes (%)	87.5	87.5	1.0**
Vitamin D supplements (%)	14.0	50.0	
Vitamin D Intake (IU)	262.2	292.0	0.81
HBA1C (%)	7.98 ± 2.2	5.9 ±0 .17	1.0**
Serum 25(OH)D3 (ng/mL)	22.6 ± 5.0	18.6 ± 3.3	0.50#
Serum Cholecalciferol (ng/dL)	4.1 ±1.2	6 ± 2.3	0.29**
FEV % in 1 sec	63.4 ± 10.0	55.8 ± 8.8	0.58*

*Oneway ANOVA
**Fisher's Exact 2-Tailed
*ANOVA >F
Oneway ANOVA T-test
+Assumed equal variance

Table 2. Serum Cholecalciferol by hour

Group	0 hour (ng/dL)*	12 hours (ng/dL)*	24 hours (ng/dL)*	48 hours (ng/dL)*	P_{group}	P_{time}	$P_{group*time}$
Powder	4.62 ± 9.40 ^{E}	144.32± 9.40 ^{A}	107.0 ± 9.40 ^{B}	60.23± 10.80 ^{C,D}	0.071	<0.0001	0.0001
Oil	6.0± 7.66 ^{E}	80.0± 7.66 ^{B,C,D}	93.7 ± 7.66 ^{B,C}	60.05±8.32 ^{D}			

* Time points not associated by the same letter are significantly different, $p<.05$
Least Squares Means
Two-way Mixed Model Repeated Measures ANOVA

Figure 3 Cholecalciferol, least squares means, N=15 Serum cholecalciferol increased over 48hrs in both the oil and the powder groups. The group-by-time interaction was statistically significant (Pgroup*time<0.0001) in the powder group.

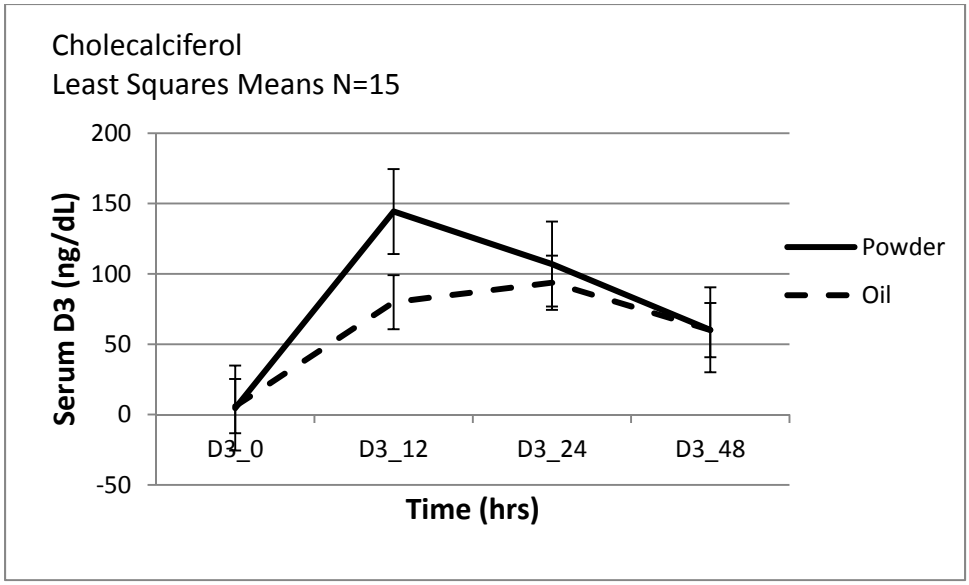

Figure 4 Mean AUC

The AUC for serum cholecalciferol was also higher in the powder group compared to the oil group (3256.5±285.5 vs 2393.73±233.1, p = 0.036).

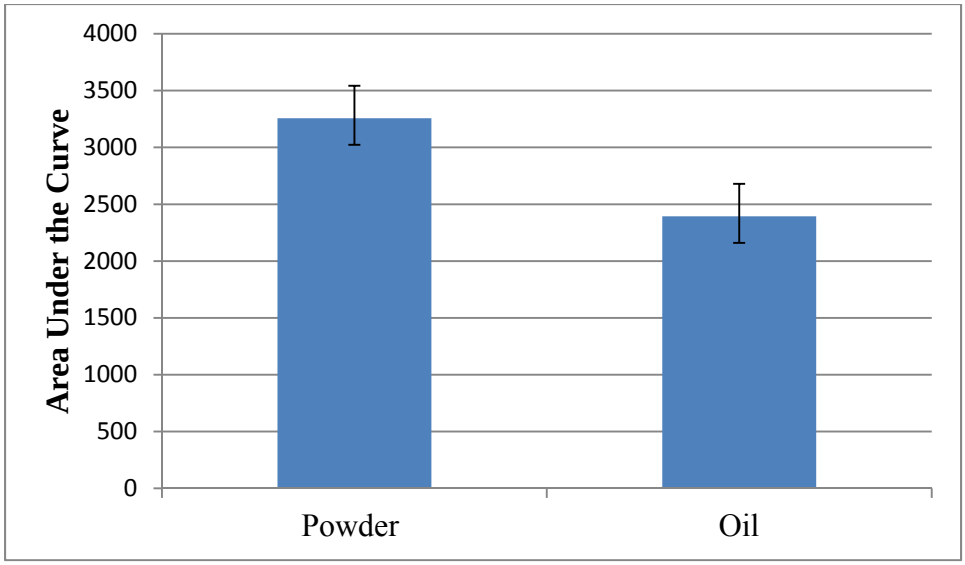

Table 3. Serum 25-Hydroxyvitamin D3 (25(OH)D3) by hour

	0 hours (ng/mL)*	12 hours (ng/mL)*	24 hours (ng/mL)*	48 hours (ng/mL)*	P_{group}	P_{time}	$P_{group*time}$
Powder	24 ±4.3	29.3 ±4.2	31.3 ±4.2	27.3 ±4.4	0.300	0.0062	0.347
Oil	18.6 ±3.4	20.7 ±3.5	22.9 ±3.4	25.6 ±3.6			

*Serum 25(OH)D by time and group (LS mean +/- SEM).
* Time points not associated by the same letter are significantly different, $p<.05$

Figure 5 Changes in Plasma 25-Hydroxyvitamin D Over Time, N=15