The Effect of Vitamin D_3 Supplementation on Physical Performance in Ambulatory Patients on Hemodialysis

by

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Abstract

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Objective: The purpose of this study was to investigate the effect of cholecalciferol (vitamin D_3) supplementation on physical performance in ambulatory hemodialysis (HD) patients.

Design and Setting: This randomized, double-blind, placebo-controlled study was conducted in three dialysis centers in eastern Alabama.

Subjects and Intervention: HD patients with serum 25-hydroxyvitamin D [25(OH)D] concentrations of less than 30 ng/mL and capable of ambulating independently were recruited for the study. Patients were randomly assigned to receive either vitamin D_3 or placebo. Patients receiving cholecalciferol were provided with one 50,000 IU pill per week for 8 weeks followed by one 50,000 IU pill every 2 weeks for the next four weeks. Patients receiving the placebo followed the same dosing schedule.

Main outcome measures: Physical performance changes pre and post intervention included: timed jump up and go test (JUG); timed 20-foot walk; timed repeated chair stand (RCS); Physical Functioning results from the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire (SF36); 25(OH)D concentrations and other biochemical values.

Results: After the intervention, the supplemented group (n=25) had significantly higher serum 25(OH)D concentrations than the placebo group (n=21) (51.8±12.9 vs. 16.0±5.2 ng/mL, respectively) and significantly lower plasma PTH concentrations than the

placebo (251.7±264.8 vs. 491.5±587.0 pg/mL, respectively). The supplemented group exhibited significantly decreased times to complete the RCS from pre- to post-intervention, but the significance was lost in the between group comparison (-2.7 seconds vs. -0.6 seconds). Scores on the SF36 questionnaire were significantly correlated with baseline serum 25(OH)D concentrations.

Conclusion: Supplementation with vitamin D can correct deficient serum 25(OH)D concentrations in HD patients. Serum 25(OH)D concentrations have a positive impact on patient well-being as measured by the SF36 questionnaire. Further research needs to be done to evaluate physical performance improvements in studies of longer duration.

Key words: Hemodialysis, Vitamin D, cholecalciferol, skeletal muscle, CKD

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List of Abbreviations

$1,25(OH)_2D$	1, 25 dihydroxyvitamin D or calcitriol
$1,25(OH)_2D_3$	1, 25 dihydroxycholecalciferol
$1,25(OH)_2D_2$	1, 25 dihydroxyergocalciferol
$24,25(OH)_2$	24,25 dihydroxyvitamin D
25(OH)D	25 hydroxyvitamin D
$25(OH)D_3$	25 hydroxycholecalciferol or calcidiol
$25(OH)D_2$	25 hydroxyergocalciferol or ercalcidiol
BMI	Body Mass Index
CKD	Chronic Kidney Disease
D_2	Ergocalciferol
D_3	Cholecalciferol
DBP	Vitamin D Binding Protein
DEXA	dual-energy x-ray absorptiometry
DRIP	Vitamin D Receptor Interacting Protein
ESRD	End Stage Renal Disease
FGF23	Fibroblast Growth Factor 23
GFR	Glomerular Filtration Rate
HD	Hemodialysis
iPTH	Intact Parathyroid Hormone (also referred to as PTH)

IU	International Unit
JUG	Timed Jump up and go test (same as TUG)
NAD^+	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
PTH	Parathyroid Hormone (also referred to as iPTH)
RCS	Repeated Chair Stand
RXR	Retinoid X Receptor
SF36	Medical Outcomes Study 36 Item Short Form Health Status Questionnaires
TUG	Timed Up and Go Test (same as JUG)
VDRE	Vitamin D Response Element

Chapter 1

Introduction

Current estimates are that approximately 40% of the US population is deficient in vitamin D (Holick, 2006). The important role of $1,25(OH)_2D_3$ in calcium and phosphorus homeostasis and bone health has long been recognized, as has the complex biosynthesis of the vitamin's active form in the kidneys which secrete this form of the vitamin into circulation. In progressive renal disease, an individual's ability to activate vitamin D in the kidneys by converting 25(OH)D to $1,25(OH)_2D$ (calcitriol) is reduced and patients often become either deficient or insufficient in active vitamin D. In addition, estimates are that 97% of hemodialysis patients are 25(OH)D deficient secondary to increased 25(OH)D excretion and to decreased 7-dehydrocholesterol production in the skin and subsequent decreased conversion to 25(OH)D (Tang, 2009). Due to the inability of the kidneys to activate 25(OH)D, treatment recommendations for hemodialysis (HD) patients are to provide calcitriol or a vitamin D analog; however, no protocols provide for the correction of the 25(OH)D deficiency.

Discovery in the last 25 years that extra-renal cells are capable of activating 25(OH)D and using this form of the vitamin within the cell has led to further research into additional roles for vitamin D. One of these roles relates to skeletal muscle function. Patients in end stage renal disease (ESRD) often exhibit myopathy that affects their ability to perform activities of daily living. Moreover, muscle wasting in patients receiving hemodialysis has been correlated with low serum 25(OH)D concentrations,

(Boudville, Inderjeeth, Elder, & Glendenning, 2010; Gordon, Sakkas, Doyle, Shubert, & Johansen, 2007; Johansen, Shubert, Doyle, Soher, Sakkas, & Kent-Braun, 2003) and myopathy has been associated with low serum 25(OH)D concentrations in individuals with normal renal function (Annweiler, Beauchet, Berrut, Fantino, Bonnefoy, Herrmann, & Schott, 2009; Bischoff-Ferrari, Dietrich, Orav, Hu, Zhang, Karlson, & Dawon-Hughes, 2004; Bunout, Barrera, Leiva, Gattas, de la Maza, Avendano, & Hirsch, 2006; Dam, von Mühlen, & Barrett-Connor, 2009; Dretakis, Tsatsanis, Fyrgadis, Drakopoulos, Steriopoulos, & Margioris, 2010; Sato, Iwamoto, Kanoko, & Satoh, 2005; Visser, Deeg, & Lips, 2003). While supplementation with vitamin D has shown mixed results in improving muscle function in healthy individuals (Bunout et al., 2006; Dhesi, jackson, Bearne, Moniz, Hurley, Swift, & Allain, 2004; Pfeifer, Begerow, Minne, Abrams, Nachtigall, & Hansen, 2000; Sato et al., 2005), no such studies have been performed in the HD population. The purpose of this 12-week, double-blind randomized clinical trial was to determine whether supplementation with vitamin D would increase serum 25(OH)D concentrations and positively impact physical performance in hemodialysis patients.

Chapter 2

Literature Review

Vitamin D deficiency is common, affecting approximately 40% of the US population; however, individuals with chronic kidney disease (CKD) and especially end stage renal disease (ESRD) are at greater risk (Holick, 2006). Muscle wasting and myopathy are common complications experienced by patients receiving hemodialysis (HD) treatment and have been correlated to low serum concentrations of vitamin D. This review of the literature covers vitamin D nomenclature; the biosynthesis, activation, regulation, and inactivation of vitamin D; regulation of serum vitamin D concentrations, impact of vitamin D sources on serum concentrations; mechanisms of vitamin D action; the functions of and effects of vitamin D supplementation, especially involving muscle morphology and function; as well as treatment recommendations for HD patients.

Nomenclature

Chronic kidney disease is a progressive disease categorized into stages by glomerular filtration rates (GFR), an index of blood filtration performed by the kidneys. Stage 1 and 2 kidney disease are defined by decreased kidney function, but are not considered chronic. Stages 3 through 5 are considered CKD with stage 3 defined as GFR between 30 and 59 mL/min; stage 4 between 15 and 29 mL/min; and stage 5 as less than 15 mL/min. Stage 5 is referred to as end stage renal disease (ESRD) and requires maintenance renal replacement therapy. This dissertation focuses on individuals receiving hemodialysis rather than an alternative renal replacement therapy (such as transplant or peritoneal dialysis).

The terminology used to describe vitamin D and its metabolites is often used inconsistently in the literature. The nomenclature used throughout this document and shown hereafter follows the recommendations of the Kidney Disease Improving Global Outcomes (KDIGO) ("KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)," 2009)

Table 2.1 Vitamin D Nomenclature

Common name	Represents Generic name	Abbreviation	If no distinction necessary
Vitamin D	Cholecalciferol Ergocalciferol	D_3 D_2	D
25-hydroxyvitamin D	Calcidiol or 25-hydroxycholecalciferol Ercalcidiol or 25-hydroxyergocalciferol	$25(OH)D_3$ $25(OH)D_2$	25(OH)D
1,25-dihydroxyvitamin D	Calcitriol or 1,25-dihyroxycholecalciferol 1,25-dihydroxyergocalciferol	$1,25(OH)_2D_3$ $1,25(OH)_2D_2$	$1,25(OH)_2D$

Biosynthesis, Activation, and Regulation of Vitamin D

The biosynthesis, activation, and regulation of vitamin D are complex, multi-step processes involving multiple organs, enzymes and metabolites. Endogenous production of vitamin D begins with cholesterol in the skin. A double bond is formed between carbons 7 and 8, generating 5,7-cholestradienol (more commonly referred to as 7-dehydrocholesterol) in the sebaceous glands of the skin. The 7-dehydrocholesterol is secreted onto the skin's surface where it is reabsorbed into the dermis. The presence of

the two double bonds in the B ring of 7-dehydrocholesterol allows absorbance of ultraviolet light (UV) in the 290-315 nm wavelengths (Jones, 2007). This UV light breaks the bond between the 9 and 10 carbons in the B ring converting 7-dehydrocholesterol to previtamin D_3 or precalciferol. Within two to three days, previtamin D_3 is thermally isomerized to vitamin D_3 (also referred to as cholecalciferol or calciol). The fat soluble vitamin D_3 diffuses from the skin into the blood where it binds with α-2 globulin vitamin D-binding protein (DBP or transcalciferin), which is synthesized in the liver (Gropper, Smith, & Groff, 2005).

In addition to endogenous production of D_3, both D_2 and D_3 can be acquired through the diet. D_2, also called ergocalciferol, is the form of vitamin D synthesized by selected plants while cholecalciferol is synthesized by animals. D_2 differs from D_3 by the presence of a double bond between carbons 22 and 23, and a methyl side chain on carbon 24. Supplements and food fortifications use both D_2 and D_3 forms (Gropper et al., 2005). The biosynthesis and degradation of D_2 and D_3 vary insignificantly. As a result, both forms will be referred to as vitamin D unless a distinction in function is necessary.

Endogenously synthesized vitamin D binds to DBP in the blood and is transported to the liver. Dietary vitamin D diffuses as part of a micelle into small intestinal cells where it is incorporated into chylomicrons which enter the lymphatic system. Chylomicrons are transported through the lymphatic system, enter the blood via the thoracic duct and are taken up by the liver (Gropper et al., 2005). Serum vitamin D concentrations are low as the vitamin is quickly taken up by the liver for further activation, or by adipocytes for storage (Jones, 2007).

In the liver, vitamin D is hydroxylated at carbon 25 to form 25-hydroxyvitamin D, abbreviated as 25(OH)D. This reaction can be catalyzed by several different enzymes of the cytochrome P450 family, including the mitochondrial enzyme CYP27A1 and the microsomal enzymes CYP2R1, CYP3A4, and CYP2C11 (the first two being more efficient and important in vitamin D synthesis in humans) (Prosser & Jones, 2004; Strushkevich, Usanov, Plotnikov, Jones, & Park, 2008). These CYP enzymes require the presence of ferredoxin reductase and ferredoxin to enable the reducing equivalents of NADPH to be transferred as part of the hydroxylation reaction. After hydroxylation, 25(OH)D is secreted by the liver into the blood and transported bound to DBP (Jones, 2007).

In the kidneys, the cell-surface receptors megalin and cubulin facilitate endocytosis of 25(OH)D into the proximal tubular cells. There, 25(OH)D is hydroxylated at carbon 1 by a 1α-hydroxylase enzyme to form the physiologically active 1α-25-dihydroxyvitamin D, abbreviated as $1,25(OH)_2D$. Similar to the liver CYP enzymes, this 1α-hydroxylase enzyme is comprised of three proteins – the mitochondrial cytochrome P450 enzyme (CYP27B1), ferredoxin, and ferrodoxin reductase. The kidneys secrete $1,25(OH)_2D$ into the blood where it circulates bound to DBP. The kidneys are considered the exclusive source of circulating $1,25(OH)_2D$ (Jones, 2007).

In the last 20 years, the 1α-hydroxylase enzyme, vitamin D receptor (VDR), and megalin/cubulin cell receptors have been found in many extra-renal cells, such as macrophages, skin, intestine, colon, prostate, breast, brain, β-islet cells, muscle, and bone. These cells are capable of hydroxylating the carbon 1 of 25(OH)D to generate the physiologically active $1,25(OH)_2D$. Under normal physiological conditions, these cells do

not secrete 1,25$(OH)_2$D into circulation, rather, the 1,25$(OH)_2$D is used in an autocrine/paracrine fashion within the cells (Jones, 2007).

Inactivation of Vitamin D and 1,25$(OH)_2$D

There are two catabolic pathways responsible for the degradation of vitamin D, 25(OH)D, and 1,25$(OH)_2$D. Both pathways use the same cytochrome P450 CYP24A1 enzyme complex consisting of CYP24A1, ferredoxin, ferrodoxin reductase, and NADPH. This enzyme complex catalyzes a 24-hydroxylation reaction inactivating both 25(OH)D and 1,25$(OH)_2$D to form 24,25$(OH)_2$D and 1,24,25$(OH)_3$D, respectively. The enzyme is 10-fold more efficient at catalyzing the reaction involving 1,25$(OH)_2$D than 25(OH)D. However, since the plasma concentration of 25(OH)D is 1000 times greater than that of 1,25$(OH)_2$D, the 24,25$(OH)_2$D can be readily measured in plasma. The 24,25$(OH)_2$D metabolite is further degraded to calcitroic acid, the primary excretory metabolite. Although the transport mechanism in the blood is not fully understood, calcitroic acid is transported to the liver and excreted in bile. These inactive metabolites have little affinity for DBP, thus they do not remain in the plasma for very long (Jones, 2007).

Regulation of Serum Vitamin D Concentration

There is little vitamin D in circulation. It is rapidly hydroxylated in the liver to 25(OH)D with little regulation. The tight regulation of circulating 1,25$(OH)_2$D occurs at the hydroxylation reaction of carbon 1. This hydroxylation is tightly controlled in a feedback loop comprised of serum concentrations of calcium, phosphorus, and vitamin D metabolites mediated by parathyroid hormone (PTH) which is secreted from the parathyroid gland (PTG). The PTG has chemoreceptors on its membrane which are sensitive to the plasma concentrations of both Ca^{2+} and phosphate (PO_4^{3-}). If plasma

calcium or phosphate concentrations are low, PTH is secreted. PTH is the principal activator of gene expression of the 1α-hydroxylase enzyme (CYP27B1) in the kidneys. The PTH binds to its receptor in the renal proximal tubular cell membrane and through cAMP and phosphatidylinositol 4,5-bisphosphate signals transcriptional upregulation of CYP27B1 protein synthesis, thereby, increasing the rate of synthesis of $1,25(OH)_2D$. As the concentration of $1,25(OH)_2D$ increases, its presence provides negative feedback that downregulates transcription of CYP27B1 hydroxylase, thereby reducing the rate of synthesis of $1,25(OH)_2D$. This downregulation occurs through direct interaction of $1,25(OH)_2D$ with upstream elements in the promoter region of the CYP27B1 gene, as well as through inhibition of PTH secretion (Prosser & Jones, 2004).

Vitamin D inactivation is also affected by fibroblast growth factor 23 (FGF23), a protein produced by osteoblasts and osteocytes in the bone that inhibits 1α-hydroxylase activity. Klotho is a cofactor essential for FGF23 action. As a result of the inhibition of 1α-hydroxylase, synthesis of $1,25(OH)_2D$ is decreased (Tang, 2009). In addition, these proteins (Klotho and FGF23) increase the catabolic activity of CYP24A1 to promote degradation of vitamin D, 25(OH)D, and $1,25(OH)_2D$ (Jones, 2010).

In macrophages, γ-interferon, a cytokine, upregulates CYP27B1, increasing the rate of synthesis of $1,25(OH)_2D$. However, regulation of CYP27B1 in other extra-renal cells is poorly understood (Prosser & Jones, 2004).

In addition to downregulation of CYP27B1 expression, $1,25(OH)_2D$ upregulates transcription of the 24-hydroxylase CYP24A1. This upregulation increases the amount of CYP24A1, which subsequently increases the rate of degradation of both $1,25(OH)_2D$ and 25(OH)D. The CYP24A1 enzyme is found in kidneys and all vitamin D target tissues. In

contrast, it is not found in the liver or osteoclasts. In the kidneys, this upregulation of the CYP24A1 occurs constitutively providing regular degradation of vitamin D and $1,25(OH)_2D$. In the extra-renal vitamin D target cells, the CYP24A1 is one of many vitamin D-dependent genes. It is expressed concurrently with these other genes, thereby creating an autoregulatory attenuation of the $1,25(OH)_2D$ hormonal signal (Prosser & Jones, 2004).

Impact of Vitamin D Sources on Serum Concentrations

The few foods that contain vitamin D include oily fish (salmon, mackerel, and herring) and liver oils from fish (cod, tuna, shark). In the US, dairy products are often fortified with vitamin D (either D_2 or D_3). However, the amounts of vitamin D are small; sufficient to prevent rickets, but not sufficient for overall good health (Holick, 2008). Given that vitamin D is a fat soluble molecule, intestinal absorption is improved in the presence of fat. However, it is unclear whether there is an optimal level of fat needed with consumption of the vitamin for promoting vitamin D absorption (Ross, 2011).

The sun is the best source for endogenous vitamin D production. However, the use of sunscreens limits or eliminates the ability of the skin to produce previtamin D from 7-dehydrocholesterol. The presence of melanin in the skin, responsible for skin pigmentation, also limits the skin's ability to produce previtamin D. Thus, individuals with darker skin are at greater risk of vitamin D deficiency (Holick, 2008).

Also, the zenith angle of the sun is important for vitamin D production. During the early morning and late afternoon hours, the ozone absorbs the UVB radiation and very little vitamin D can be produced by the skin during this time. In addition, as one moves further from the equator during the winter months, the zenith angle of the sun

reduces the UVB radiation that reaches the earth. As a result, individuals living north of the 35° latitude are not able to produce vitamin D between October and March. Further north, and individuals may not be able to produce vitamin D between September and April (Holick, 2008).

In circulation, 25(OH)D is the primary metabolite and is found bound to DBP in concentrations of 10-80 ng/mL or roughly 1000 times greater than the amount of circulating $1,25(OH)_2D$ (Holick, 2009). The half-life of 25(OH)D is 10 days to 3 weeks while that of $1,25(OH)_2D$ is 4 to 6 hours (Holick, 2009). Serum 25(OH)D concentrations are used to assess a person's vitamin D status.

Although experts do not agree on the optimal serum concentration of vitamin D for overall health, there is general agreement on deficient and insufficient concentrations. Deficiency is defined as serum concentration of 25(OH)D less than 20 ng/mL and insufficiency as 21 to 29 ng/mL. This definition is due to vitamin D's effect on PTH. An observational study performed by Chapuy et al. (Chapuy, Preziosi, Maamer, Arnaud, Galan, Hercberg, & Meunier, 1997) determined that serum PTH concentration plateaus at its lowest concentration when $25(OH)D_3$ is between 31 and 40 ng/mL. Malabanan et al. (Malabanan, Veronikis, & Holick, 1998) supplemented healthy adults who had serum 25(OH)D concentrations between 11 and 25 ng/mL with 50,000 IU units of vitamin D once per week and tracked changes in serum PTH concentrations. Serum PTH concentration showed significant decreases as the serum concentration of vitamin D increased for those with serum 25(OH)D between 11 and 19 ng/mL. Serum PTH concentrations for those with 25(OH)D concentrations greater than 20 ng/mL did not show any significant changes.

Data from the National Health and Nutrition Examination Survey (NHANES) III indicated that greater than 40% of the US population is vitamin D deficient or insufficient (Holick, 2006). Individuals with ESRD are at a much greater risk of vitamin D deficiency than is the general public for several reasons. Individuals with proteinuria, one characteristic of ESRD, have decreased uptake of the 25(OH) D/DBP into the renal tubules and increased vitamin D excretion in the urine. Additionally, the uremia associated with ESRD decreases the skin's ability to convert 7-dehydrocholesterol to previtamin D. As a result, it is estimated that vitamin D deficiency/insufficiency may be as high as 86% and 97% in predialysis and dialysis patients, respectively (Tang, 2009). Further, plasma $1,25(OH)_2D$ concentrations are decreased as 1α-hydroxylase activity is depressed due to reduced renal function, hyperuricemia, metabolic acidosis and increased concentrations of FGF23 (Tang, 2009). The most reliable way to improve serum 25(OH)D concentrations is through oral supplementation. For every 100 IU of vitamin D ingested, serum 25(OH)D concentration increases by approximately 1 ng/mL. However, if serum 25(OH)D concentrations are below 20 ng/mL, the body is more efficient at absorbing the vitamin D and serum concentrations will rise by approximately 2 to 3 ng/mL for every 100 IU consumed (Holick, 2009). Thus, the estimated supplementation dose to raise serum 25(OH)D concentration above 30 ng/mL is 1800 to 5000 IU/day in a hemodialysis patient (Jones, 2010).

Al-Aly et al. (Al-Aly, Qazi, González, Zeringue, & Martin, 2007) assessed new referrals to an outpatient nephrology clinic for 25(OH)D deficiency and PTH elevation. There were 66 patients out of 74 assessed (89%) who met the inclusion criteria of serum 25(OH)D concentrations less than 30 ng/mL and serum PTH concentration greater than

110 pg/mL. All participants received 50,000 IU of ergocalciferol once a week for 12 weeks followed by 50,000 IU once a month for the following 3 months. The serum concentration of 25(OH)D increased significantly from an average of 16.6 ng/mL to 27.2 ng/mL for the entire cohort. However, 16 patients exhibited no increase in serum 25(OH)D concentrations and only 12 patients achieved a concentration greater than 40 ng/mL Reasons for non-responsiveness were not clear, although noncompliance in taking the supplement may have been a contributing factor.

Saab et al. (Saab, Young, Gincherman, Giles, Norwood, & Coyne, 2007) studied the effect of ergocalciferol administration in 131 HD patients receiving 50,000 IU doses of ergocalciferol, monthly for 6 months. Baseline characteristics indicated that 92% of the patients were 25(OH)D insufficient (serum concentrations <30 ng/mL) and 51% were deficient (< 15 ng/mL). The mean serum 25(OH)D concentration at baseline was 16.9 ng/mL and increased significantly to 53.6 ng/mL by the end of the study. In addition, all of the participants had serum 25(OH)D concentrations above 15 ng/mL and 94% had concentrations above 30 ng/mL.

There is controversy concerning the relative efficacy of ergocalciferol supplementation compared to cholecalciferol. The following two studies demonstrate that cholecalciferol is more efficacious than ergocalciferol at increasing serum 25(OH)D concentrations. Armas et al. (Armas, Hollis, & Heaney, 2004) studied the efficacy of these two forms of vitamin D in 30 healthy individuals who were randomized to receive either no treatment; one 50,000 IU dose of ergocalciferol; or one 50,000 IU dose of cholecalciferol. Serum 25(OH)D concentrations were measured on days 0, 1, 3, 5-7, 14, and 28. The two groups receiving vitamin D showed a significant increase in serum

vitamin D on day 1 from baseline, however, these two groups were not significantly different from each other. On day 3, both groups showed significant decreases in serum 25(OH)D concentrations when compared to day 1. Again, these two groups were not significantly different from each other. However, the area under the curve (AUC) of the serum concentration against time was calculated for the 3 groups for 28 days. The AUC for D_3 was significantly greater than that for D_2 (204.7 ng·d/mL vs 60 ng·d/mL, respectively). This difference suggests that cholecalciferol has a three-fold greater potency than ergocalciferol at increasing serum 25(OH)D concentrations.

Binkely et al. (Binkley, Gemar, Engelke, Gangnon, Ramamurthy, Krueger, & Drezner, 2011) also evaluated the efficacy of D_2 vs D_3 dosing in 64 community dwelling adults over the age of 65 years. The participants were randomized into four groups to receive the following interventions for 12 months: 1600 IU daily of D_2; 1600 IU daily of D_3; 50,000 IU monthly of D_2; or 50,000 IU monthly of D_3. Serum 25(OH)D concentrations increased significantly with all interventions. Frequency of dosing did not generate a significant difference in serum levels. D_3 supplementation resulted in a significantly greater increase in serum concentration than did D_2 (9.2 vs. 6.1 ng/mL, respectively, for daily dosing; 8.9 vs. 3.6 ng/mL, respectively, for monthly dosing). The average increase in serum 25(OH)D concentrations attained per 100 IU of daily vitamin D_3 and D_2 was 0.58 and 0.38 ng/mL, respectively - there was substantial variability between individuals as to response to the dosing. This variability was not deemed to be related to either baseline serum concentrations or to compliance.

Mechanisms of 1,25$(OH)_2$D Action

Vitamin D has both genomic and non-genomic activity affecting several bodily functions including serum calcium homeostasis, cell proliferation, differentiation, apoptosis, and muscle function among other roles. Current estimates are that 1,25$(OH)_2$D participates in the genomic regulation of approximately 5% of genes or 300-800 genes. The majority of 1,25$(OH)_2$D produced by the kidneys circulates bound to DBP. However, primary target cells (intestine, kidney, parathyroid glands and bone) do not have the megalin/cubulin cell surface receptors responsible for facilitating endocytosis of the vitamin D/DBP complex. As a result, it is free 1,25$(OH)_2$D that enters target cells through diffusion and binds to the VDR in the nucleus. The liganded VDR complex targets 1,25$(OH)_2$D dependent genes and interacts with a specific nucleotide sequence upstream of the gene. This region is known as the VDRE. The binding of 1,25$(OH)_2$D to the VDR causes a conformational change that allows the formation of a heterodimer with the retinoid X receptor (RXR). Additionally, several other proteins interact with the vitamin D receptor interacting protein (DRIP) which positions the VDR's zinc finger regions to interact with DNA to activate gene transcription either through initiation of or an increased rate of gene transcription (Ceglia, 2009; Jones, 2007).

The extra-renal 1α-hydroxylating target cells (brain, muscle, macrophages) operate in a slightly different manner. These cells have the megalin/cubulin cell receptors to facilitate the endocytosis of the vitamin D/DBP complex. Thus, these cells readily take up 25(OH)D as well as 1,25$(OH)_2$D. As these cells have the CYP27B1 enzyme, the cells will generate their own supply of 1,25$(OH)_2$D from 25(OH)D. The presence of the CYP27B1 enzyme in these cells is hypothesized to enable the cells to achieve higher

intracellular concentrations of $1,25(OH)_2D$ and that this higher concentration may be necessary to initiate gene transcription in these cells when compared to primary target cells (Jones, 2010).

The non-genomic mechanism(s) by which vitamin D functions are not clearly understood. However, rapid, non-transcriptional responses initiated by vitamin D in some cells cannot be explained by the slow, genomic pathway. It is possible that 25(OH)D binds to a novel, unidentified cell membrane receptor responsible for initiating second messenger signaling. Or, it may be that the VDR is translocated to and embedded in the cell membrane where it behaves as a cell membrane receptor (Ceglia, 2008).

Overview of Vitamin D Functions

Serum Calcium Homeostasis

Vitamin D along with PTH functions in the regulation of serum calcium concentrations. The PTG is sensitive to low serum concentrations of calcium, phosphorous, and $1,25(OH)_2D$, secreting PTH when serum concentrations of these nutrients are low. The increased serum $1,25(OH)_2D$ concentrations resulting from increased PTH act on: enterocytes, increasing absorption of both calcium and phosphorous; the bone, increasing resorption of calcium and phosphorus; and the kidneys, increasing reabsorption of urinary calcium. The resulting increase in calcium provides negative feedback to the PTG to inhibit PTH secretion.

Non-Skeletal, Non-Muscular Functions

Many tissues in the body have VDRs and express the 1α-hydroxylase enzyme including the brain, prostate, breast, colon, monocytes, and macrophages. The $1,25(OH)_2D$ produced in these tissues/cells controls many genes responsible for cell

proliferation, differentiation, apoptosis, and angiogenesis. Cell proliferation is reduced and terminal differentiation is induced – both in normal and cancer cells in response to the cellular presence of vitamin D. Monocytes and macrophages that have been exposed to a foreign entity upregulate both the VDR and the 1α-hydroxylase enzyme genes. The resulting increase in $1,25(OH)_2D$ drives the innate immune response through an increase in the synthesis of cathelicidin, a peptide capable of destroying various infectious agents. At serum concentrations below 20 ng/dL, 25(OH)D is not capable of initiating this response (Holick, 2007). Other activities of $1,25(OH)_2D$ include the inhibition of renin synthesis (thereby reducing blood pressure); increased insulin production (reducing the risk of hyperglycemia); and increased myocardial contractility (Holick, 2007).

Individuals living at higher latitudes throughout the world are at increased risk of vitamin D deficiency. Vitamin D deficiency has been link with increased risk of death and several chronic diseases including; cancer (Hodgkin's lymphoma, colon, pancreatic, prostate, ovarian, and breast); autoimmune diseases; (multiple sclerosis, rheumatoid arthritis, Crohn's disease and type 1 diabetes); hypertension and cardiovascular disease; schizophrenia; and depression (Holick, 2007).

Muscle Function

Vitamin D is involved in many genomic functions necessary for contraction in the myoblast, including the initiation of gene transcription of the calcium binding proteins calbindin D9K and calmodulin. Calbindin D9K is responsible for calcium transport within the cell. Calmodulin mediates many cellular activities including inflammation, apoptosis, calcium transport and storage, and smooth muscle contraction. Increased phosphate uptake into skeletal muscle is accelerated through vitamin D genomic activity.

Increased cytosolic concentrations of both calcium and phosphorus are important for muscle contraction.

One non-genomic activity of vitamin D related to calcium uptake includes the opening of voltage-gated calcium channels to increase calcium influx into the cell. Increased concentrations of intracellular calcium and phosphate are critical for muscle contraction. In addition, vitamin D activates MAPK pathways which initiate myogenesis, cell proliferation, differentiation, or apoptosis. However, the mechanisms by which vitamin D affects these pathways are not fully understood (Ceglia, 2008).

Vitamin D Related Myopathy and Physical Performance

Healthy Individuals

Adults with low serum 25(OH)D concentrations display type II muscle fiber atrophy (Ceglia, 2008). Type II muscle fibers, referred to as fast twitch fibers, are the first to be used for sudden, rapid movement such as stabilization when balance is lost or standing up from a seated position. In addition, muscle biopsies from 25(OH)D deficient individuals show enlarged interfibrillar spaces and infiltration of fat, fibrosis and glycogen granules (Ceglia, 2008). However, vitamin D supplementation has been shown to improve the type II atrophy.

Sato et al. (2005) studied 96 elderly women with post stroke hemiplegia over 2 years. All of the women started the study with deficient serum 25(OH)D concentrations of less than 10 ng/mL. Half of the women were supplemented with 1,000 IU of vitamin D daily. Serum concentrations of both 25(OH)D and $1,25(OH)_2D$ were improved in the supplemented group. In addition, there was a 59% reduction in falls in the supplemented group.

In adults, low serum 25(OH)D has been associated in some, but not all, studies with proximal muscular weakness that results in decreased ability to perform activities of daily living such as walking up stairs, standing up from a seated or squatting position, and in lifting objects (Annweiler et al., 2009; Bischoff-Ferrari et al., 2004; Dam et al., 2009; Dretakis et al., 2010; Visser et al., 2003). Bischoff-Ferrarri et al. (2004) analyzed the physical performance of 4,100 men and women over 60 years of age from the NHANES III survey based on 25(OH)D status. Individuals with serum concentrations between 9 ng/mL and 37 ng/mL performed 8-foot walk tests and repeated sit from a chair to stand tests. A significant positive association was found between physical performance and serum 25(OH)D concentrations. This association was independent of the individual's physical activity level.

Visser et al. (2003) followed approximately 1,000 men and women over 65 years of age for three years as part of the Longitudinal Aging Study Amsterdam (LASA). Individuals with serum 25(OH)D concentrations less than 10 ng/mL had a 2.57 fold greater risk of developing sarcopenia based on the results of a hand grip test. Of the 300 individuals who underwent dual-energy x-ray absorptiometry (DEXA) scans, those with serum 25(OH)D concentrations less than 10 ng/mL had a 2.14 times greater risk of developing sarcopenia than those with serum 25(OH)D concentrations greater than 20 ng/mL. Dretakis et al. (2010) found a significant positive correlation between serum 25(OH) concentrations and quadriceps muscle strength in 48 community dwelling individuals over 65 years of age.

Dam et al. (2009) studied 769 community dwelling men and women with an average age of 74.5 years to assess physical performance as a function of serum

25(OH)D concentrations. Participants performed timed up and go (TUG), grip strength (GS), and repeated chair stand (RCS) tests at baseline and 2.5 years later. The women with the lowest quartile of serum 25(OH)D (<80 nmol/L or <32 ng/mL), but not the men, showed a significantly greater decrease in performance on the TUG and RCS tests at follow up compared to women in the highest quartile of serum 25(OH)D (115-337.5 nmol/L or 46-135 ng/mL) - approximately a three-fold effect (decrease of 25% vs. 8%, respectively). Further analysis showed that the women with insufficient 25(OH)D levels (<75 nmol/L or <30 ng/mL) had a mean decrease in performance of 39% versus 10% in women with sufficient levels.

In contrast, Annweiler et al. (2009) studied 440 women from the EPIDOS study (a community-dwelling, observational, prospective cohort study designed to evaluate risk of hip fractures in women 75 years or older in five French cities). The women performed two strength measure procedures to determine the maximal isometric voluntary contraction (MVC) of the quadriceps and handgrip. The women were grouped based on serum 25(OH)D concentrations: < 15 ng/mL; 15-30 ng/mL; and >30 ng/mL. There was no difference in quadriceps strength among the three groups (174.9, 175.9 and 173.4 Newton per square meter, respectively). Similarly, handgrip strength was 56.1, 57.1 and 61.1 Newton per square meter, respectively). The authors found that older age, comorbidities, and poor renal function were associated with muscle weakness, while higher BMI and regular physical activity were associated with greater muscle strength.

Supplementation with vitamin D has been shown to improve physical performance in individuals with low serum 25(OH)D concentrations (Bunout et al., 2006; Dhesi et al., 2004; Pfeifer et al., 2000). Pfeiffer et al. (2000) studied 148 women over 70

years of age to determine if 8 weeks of calcium with vitamin D supplementation was more effective than calcium alone in preventing falls. The women, all of whom had serum 25(OH)D concentrations less than 20 ng/mL, received either 1,200 mg calcium with or without 800 IU vitamin D. The women were followed for one year to determine fall risk. The women receiving the vitamin D supplement had increases in serum 25(OH)D concentrations of 72% and a decrease in body sway of 9% (a risk factor for falls). Additionally, the mean number of falls was 0.45 for the calcium only supplemented group and 0.24 for the dual supplementation group.

Bunout et al. (2006) studied the effects of vitamin D supplementation when combined with resistance training on physical performance in 96 individuals over the age of 70 years. The individuals had serum 25(OH)D concentrations of less than 16 ng/mL and were randomized to one of four groups: trained with 400 IU vitamin D plus 800 mg calcium daily; trained with 800 mg calcium daily; untrained with 400 IU vitamin D plus 800 mg calcium daily; or untrained with 800 mg calcium daily. At the end of 9 months, there was a significant improvement from 12.4 ng/mL to 25.8 ng/mL in serum 25(OH)D concentrations in both groups treated with the vitamin D. No improvement in serum 25(OH)D concentrations was seen in the individuals receiving calcium alone. Trained individuals had significant improvements in quadriceps muscle strength and TUG tests; however, improvements were significantly better in those receiving the vitamin D. Also, individuals supplemented with vitamin D had significantly higher gait speeds than those who did not receive the vitamin D.

Dhesi et al. (2004) studied the effect of a single intramuscular injection of 600,000 IU ergocalciferol versus placebo on muscular function in 139 individuals over

the age of 65 years with serum 25(OH)D concentrations less than 12 ng/mL and a history of falls. At 6 months, the serum 25(OH)D concentrations increased significantly from 10.4 ng/mL to 17.5 ng/mL in the treated group versus an increase from 10.0 ng/mL to 12.6 ng/mL in the placebo group. The increase in the placebo group was attributed to seasonal increase in sun exposure. The participants were evaluated on four physical function tests including: AFPT (Aggregate Functional Performance Time; a combined time of four activities including a 50-ft walk, rising from a chair and walking 50 ft, ascent and descent of 13 steps); CRT (a measure of four choice reaction time as measured by computer); postural stability; and quadriceps strength. Performance in the AFPT deteriorated by 6.6 seconds in the placebo group while improving 2.0 seconds in the treatment group. The treatment group had an improvement of 0.41 seconds on the CRT test. Postural sway worsened in the placebo group by 3% yet improved by 13% in the treatment group. Both groups showed deterioration in quadriceps muscle strength that was not significantly different between groups.

Individuals with ESRD

Low serum 25(OH)D concentrations have a similar effect on physical performance and function in those with ESRD as that found in individuals with adequate renal function (Boudville et al., 2010; Gordon et al., 2007; Johansen et al., 2003). Johansen et al. (2003) compared the muscle strength, muscle quality, and physical function in 38 HD patients to 19 healthy, sedentary individuals. HD patients were weaker, less active, and walked more slowly than their healthy peers. The authors attributed this difference to muscle atrophy in the HD patients; however, serum vitamin D concentrations were not measured.

Gordon et al. (2007) compared the lower limb muscle size and strength in 49 HD patients receiving calcitriol or paricalcitol (a vitamin D analog) versus 30 HD patients who were receiving no treatment. The treated patients had larger thigh muscles and were stronger in measures of isotonic/isokinetic strength, and maximum voluntary contraction than the untreated patients. Treated patients were also able to walk 20 feet, climb a flight of stairs, and perform the get up and go test more quickly than were the non-treated patients. No significant differences in outcome measures were observed between the groups receiving calcitriol and paricalcitol. Study findings, however, have been criticized as the authors were not aware of duration or history of vitamin D treatment in the study population. It is likely that those patients not receiving treatment were deficient, yet treatment was not given for other reasons related to their disease state (such as hypercalcemia, hyperphosphatemia, or low PTH). One additional flaw not addressed by the authors was that no serum 25(OH)D or $1,25(OH)_2D$ concentrations were reported for the patients. Thus, it is not possible to determine the vitamin D status of any of the patients.

Boudville et al. (2010) evaluated the risk of falls of 25 HD patients in relation to serum 25(OH)D, $1,25(OH)_2D$, and intact PTH (iPTH) concentrations. Muscle strength, Falls-Screen test, and the Modified Barthel Index were positively correlated with serum 25(OH)D but not with serum $1,25(OH)_2D$ or PTH concentrations. Calcitriol treatment was given to 16 of the 25 patients. No one received ergocalciferol or cholecalciferol supplementation.

Treatment Recommendations for HD Patients

Two organizations have developed evidence-based clinical practice guidelines for the management of all stages of chronic kidney disease (CKD). The National Kidney Foundation Kidney Disease Outcome Quality Initiative (KDOQI) is based in the US and has been providing guidelines since 1997. ("K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease," 2003) In 2003, a non-profit, international foundation was established known as KDIGO (Kidney Disease: Improving Global Outcomes). KDIGO's mission is to "improve the care and outcomes of kidney disease patients worldwide through promoting coordination, collaboration, and integration of initiatives." (Eknoyan, Lameire, Barsoum, Eckardt, Levin, Levin, & Wang, 2004) These two organizations have similar recommendations with differences primarily due to available research findings at the time of the publications (KDIGO in 2009 and KDOQI in 2003) as well as the financial and insurance environment in various countries as it impacts medical treatment.

Hemodialysis was not considered a viable treatment for chronic kidney disease until the late 1960's after Dr. Belding Scribner developed the first reusable vascular access (arterial venous fistula or AV fistula). However, in the US, it was not until the 1972 passage of HR1when Medicare expanded coverage to individuals with chronic kidney failure of any age that HD became a commonly available treatment option for ESRD. As a result, long term complications of HD and ESRD are only now becoming known and investigated. One such complication is referred to as CKD – mineral and bone disorder (CKD-MBD). KDIGO defines CKD-MBD as a "systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of

the following: abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism; abnormalities in bone turnover, mineralization, volume, linear growth, or strength; or vascular or other soft-tissue calcification." (Moe, Drueke, Cunningham, Goodman, Martin, Olgaard, Eknoyan, 2006) It is within this context that recommendations regarding administration of vitamin D and its analogs have been developed based on the best available research evidence.

KDOQI recommendations state that HD patients with serum PTH concentration above 300 pg/mL should receive 1,25$(OH)_2$D (calcitriol) or an analog (alfacalcidol, paricalcitol, doxercalciferol, or 22-oxacalcitriol) to reduce serum PTH concentrations to the target range of 150-300 pg/mL. Effective dosing (and choice) of these medications is a complex titration based on the changes in plasma concentrations of PTH and serum concentrations of calcium and phosphorus. These medications suppress the PTG and inhibit secretion of PTH while increasing intestinal absorption of calcium and phosphorus. Thus, the desired outcome is a balance of appropriate suppression of PTH secretion (such that plasma concentrations are within the target range) while avoiding hypercalcemia or hyperphosphatemia. There are no recommendations regarding the administration of ergocalciferol or cholecalciferol, or the monitoring of 25(OH)D status in HD patients. (K/DOQI, 2003)

KDIGO recommendations expand upon those of KDOQI and include opinions regarding 25(OH)D status, monitoring, and supplementation based on a review of the research evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. This approach assigns a level 1 (strong recommendation) or level 2 (weak or discretionary recommendation) recommendation

with a grade of A (high), B (moderate), C (low), or D (very low) based on the quality of the supporting evidence. Based on the GRADE approach, KDIGO recommendations state "In patients with CKD stages 3-5, we suggest that calcidiol (25[OH]D) might be measured, with repeated testing determined by baseline values and therapeutic interventions (2C). We suggest that vitamin D deficiency and insufficiency be corrected using treatment strategies recommended for the general population (2C)."(KDIGO , 2009)

Due to the biochemical abnormalities of ESRD, there are many biochemical measures that have goal values that are different than those for individuals with normal renal function. Table 2.2 compares the normal reference range versus the targeted range for individuals on HD. In some cases, the target range used in the clinics in which this research was performed is different than KDOQI recommendations. In these cases, there are two ranges: the first is the KDOQI recommendation and the second is the FMC (Fresenius Medical Care) recommendation. The FMC goal for hemoglobin is lower than the KDOQI recommendation based on current reimbursement regulations of Centers for Medicare & Medicaid Services (CMS). The FMC target range for phosphorus is lower than the KDOQI recommendation based on more recent studies indicating that phosphorus levels closer to the normal reference range increase health and longevity in HD patients.

Table 2.2 Comparison of Normal to HD Blood Biochemical Reference Ranges

Biochemical Measure	Normal Reference Range	HD Reference Range
Albumin (g/dL)	3.5 – 5.0	4.0 – 5.0
iPTH (pg/mL)	10 – 60	150 – 300
Hemoglobin (g/dL)	F: 12.1 – 15.6 M: 14.6 – 17.5	KDOQI: 11.0 – 12.0 FMC: 10.0 – 11.0
Corrected calcium (mg/dL)	8.4 – 10.2	8.4 – 9.5
Phosphorus (mg/dL)	2.3 – 4.3	KDOQI: 3.5 – 5.5 FMC: 3.0 – 5.5

Research Needs and Justification

Up to 97% of dialysis patients may be vitamin D deficient or insufficient (Tang, 2009). Observational studies indicate that similar to individuals with normal renal function, physical performance is correlated with serum 25(OH)D concentrations in individuals with impaired renal function (Boudville et al., 2010; Gordon et al., 2007; Johansen et al., 2003). Randomized controlled trials (RCTs) providing supplemental vitamin D have shown that improving 25(OH)D levels may improve muscle function and physical performance in individuals with normal renal function (Bunout et al., 2006; Dhesi et al., 2004; Pfeifer et al., 2000). Theoretically, one would expect similar improved results in individuals with ESRD – yet, these RCTs have not been performed. The questions this study sought to answer were whether supplementation with vitamin D_3 for 12 weeks improved serum 25(OH)D concentrations and improved physical performance in ambulatory patients on hemodialysis.

Hypotheses

1. There will be a significant increase in serum 25(OH)D concentrations of the treatment participants from baseline to completion of the 12 week intervention.
2. There will be no significant difference in serum 25(OH)D concentrations of the placebo group from baseline to completion of the 12 week intervention.
3. There will be no significant differences in multiple biochemical metrics either between groups or from baseline to completion of study. These metrics include BMI and serum concentrations of albumin, PTH, phosphorus, corrected calcium, and hemoglobin.
4. There will be a significant correlation between serum concentrations of 25(OH)D and performance of a timed 20-foot walk.
5. Increased serum concentrations of 25(OH)D will predict improvement in the performance of a timed 20-foot walk.
6. There will be significant correlation between serum concentrations of 25(OH)D and performance of a TUG test.
7. Increased serum concentrations of 25(OH)D will predict improvement in the performance of a TUG test.
8. There will be significant correlation between serum concentrations of 25(OH)D and performance of a timed RCS test.
9. Increased serum concentrations of 25(OH)D will predict improvement in the performance of a timed RCS test.

10. There will be significant correlation between serum concentrations of 25(OH)D and scores on the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire.
11. Increased serum concentrations of 25(OH)D will predict improvement in scores on the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire.

Chapter 3

The Effect of Vitamin D_3 Supplementation on Physical Performance in Ambulatory Patients on Hemodialysis

Abstract

Objective: The purpose of this study was to investigate the effect of cholecalciferol (vitamin D_3) supplementation on physical performance in ambulatory hemodialysis (HD) patients.

Design and Setting: This randomized, double-blind, placebo-controlled study was conducted in three dialysis centers in eastern Alabama.

Subjects and Intervention: HD patients with serum 25-hydroxyvitamin D [25(OH)D] concentrations of less than 30 ng/mL and capable of ambulating independently were recruited for the study. Patients were randomly assigned to receive either vitamin D_3 or placebo. Patients receiving cholecalciferol were provided with one 50,000 IU pill per week for 8 weeks followed by one 50,000 IU pill every 2 weeks for the next four weeks for a total of 12 weeks. Patients receiving the placebo followed the same dosing schedule.

Main outcome measures: Physical performance changes pre- and post-intervention included: timed up and go test (TUG); timed 20-foot walk; timed repeated chair stand (RCS); Physical Functioning results from the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire (SF36); 25(OH)D concentrations and other biochemical values.

Results: After the intervention, the supplemented group (n=21) had significantly higher serum 25(OH)D concentrations than the placebo group (n=25) (51.8±12.9 vs. 16.0±5.2 ng/mL, respectively) and significantly lower plasma PTH concentrations than the placebo (251.7±264.8 vs. 491.5±587.0 pg/mL, respectively). The supplemented group exhibited significantly decreased times to complete the RCS from pre- to post-intervention, but the significance was lost in the between group comparison (-2.7 seconds vs. -0.6 seconds). Scores on the SF36 questionnaire were significantly correlated with baseline serum 25(OH)D concentrations.

Conclusion: Supplementation with vitamin D can correct deficient serum 25(OH)D concentrations in HD patients. Serum 25(OH)D concentrations have a positive impact on patient well-being as measured by the SF36 questionnaire. Further research needs to be done to evaluate physical performance improvements in studies of longer duration.

Key words: Hemodialysis, Vitamin D, cholecalciferol, skeletal muscle, CKD

Introduction

Current estimates are that approximately 40% of the US population is deficient in vitamin D.[1] The important role of $1,25(OH)_2D_3$ in calcium and phosphorus homeostasis and bone health has long been recognized, as has the complex biosynthesis of the vitamin's active form in the kidneys which secrete this form of the vitamin into circulation. In progressive renal disease, an individual's ability to activate vitamin D in the kidneys by converting 25(OH)D to $1,25(OH)_2D$ (calcitriol) is reduced and patients often become either deficient or insufficient in active vitamin D. In addition, estimates are that 97% of hemodialysis patients are 25(OH)D deficient secondary to decreased 7-

dehydrocholesterol production in the skin subsequent conversion to 25(OH)D and increased 25(OH)D excretion.[2] Due to the inability of the kidneys to activate 25(OH)D, treatment recommendations for hemodialysis (HD) patients are to provide calcitriol or a vitamin D analog; however, no protocols provide for the correction of the 25(OH)D deficiency.

Discovery in the last 25 years that extra-renal cells are capable of activating 25(OH)D and using this form of the vitamin within the cell has led to further research in additional roles for vitamin D. One of these roles relates to skeletal muscle function. Patients in end stage renal disease (ESRD) often exhibit myopathy that affects their ability to perform activities of daily living. Moreover, muscle wasting in patients receiving hemodialysis has been correlated with low serum 25(OH)D concentrations,[3-5] and myopathy has been associated with low serum 25(OH)D concentrations in individuals with normal renal function.[6-12] While supplementation with vitamin D has shown mixed results in improving muscle function in healthy individuals,[6,12-14] no such studies have been performed in the HD population. The purpose of this 12-week, double-blind randomized clinical trial was to determine whether supplementation with vitamin D would significantly increase serum 25(OH)D concentrations and would positively impact physical performance in hemodialysis patients.

Subjects and Methods

Subjects

The medical histories of HD patients were reviewed from three outpatient HD clinics. To be included in the study, HD patients had to be deficient in vitamin D as defined by a serum 25(OH)D concentration of less than 30 ng/mL when drawn in March

2012. Additional inclusion criteria included: patients had to be ambulatory and on maintenance outpatient HD, three times per week for more than three months; good compliance with treatment (not more than 2 missed treatments in the month prior to study enrollment); and adequate clearance as indicated by Kt/V ≥ 1.2. Exclusionary medical criteria included: malignancy; inability to provide informed consent; active drug user; myocardial infarction; congestive heart failure; or orthopedic or musculoskeletal limitations (amputation greater than toes; currently receiving treatment for foot or leg wound; or inability to ambulate independently). The study was approved by both the Auburn University Institutional Review Board for the Use of Human Subjects in Research and the Fresenius Medical Services Clinical Studies Department (Waltham, MA).

Subjects were randomly assigned to receive either vitamin D_3 or placebo manufactured by Bio-Tech Pharmacal, Inc. (Fayetteville, AR). Assignment to treatment was conducted by an independent faculty member at Auburn University. Study personnel and subjects were blinded to who received the vitamin versus the placebo. Participants received either one pill of 50,000 IU of cholecalciferol a week for 8 weeks followed by 50,000 IU every 2 weeks for the third month of the study or a placebo.

Physical Performance Measurements

Measurements of physical performance were conducted prior to the vitamin D/placebo supplementation and repeated at the end of the 12-week study period. Patients were timed to one hundredth of a second using a Sportline 226 stopwatch (Sportline, Yonkers, NY) while performing three different physical tests: RCS;[15] TUG;[16] and the timed 20 foot walk.[15] All tests are measured in seconds and have been validated as

substitute measures for lower extremity strength. These three physical performance assessment tests are easily administered with minimal equipment and space, thus are ideal for use in the clinical setting. The RCS test has an individual begin and end in a seated position. Upon commencement of the test, the individual stands up, sits back down and repeats this, continuously for 5 stands. The test should be completed as quickly as possible. Assistance can be allowed, but should be monitored and controlled for by the study coordinator. The TUG test begins from a seated position. The individual stands, walks 10 feet, turns, returns to the chair and sits. This test should be completed as quickly as possible. The timed 20 foot walk starts from a standing position, the individual walks 20 feet at a normal pace. In the first month of the intervention, a 3-day average step count was calculated to provide baseline activity levels (tracked with Omron pedometer HJ-720ITC manufactured by Omron Healthcare, Inc., Kyoto, Japan).

The Medical Outcomes Study 36-Item Short Form Survey (SF36),[17] a quality of life assessment tool, was completed at baseline and at completion of the intervention. The questions in the SF36 tool ask patients to characterize their perceptions of their physical and mental functioning. The questionnaire is scored on a scale of 0 to 100, representing lowest to highest perceived health. This tool has been validated for use with HD patients.[18,19]

Biochemical Measurements

Serum biochemical measurements were performed as part of standard patient care and were gathered through reviews of patient records. Monthly blood samples were drawn midweek to obtain predialysis serum concentrations of intact parathyroid hormone (PTH by chemiluminescence analysis), calcium (bichromatic analysis), phosphorus

(colorimetric analysis), albumin (bichromatic analysis), and hemoglobin (colorimetric analysis). The serum concentration of 25(OH)D (chemiluminescence analysis) was measured at both the beginning and conclusion of the 12-week intervention period. All serum analyses were conducted by Spectra Laboratory Services (Rockleigh, NJ).

Monthly calculated values included Kt/V and corrected calcium. The Kt/V is a complex natural logarithmic equation that quantifies HD treatment adequacy. K is the dialyzer clearance of urea, t is the dialysis treatment time, and V is the distribution volume of urea which is approximately equal to the total volume of water in the body. Standard patient care was followed and dialysis prescriptions (time of treatment and blood flow rate) were modified as needed to achieve delivered Kt/V ≥ 1.2. Corrected calcium was calculated as [(4.0-serum albumin g/dL) x 0.8] + serum calcium mg/dL.

Monitored medication dosages provided during HD treatments included Paricalcitol (Zemplar manufactured by Abbot Laboratories of Abbott Park, Illinois) and Epoetin Alfa (Epogen manufactured by Amgen, Thousand Oaks, California). Dosages were adjusted by Fresenius Medical Care (FMC) personnel per standard patient care protocols to achieve plasma PTH concentrations between 150 and 300 pg/dL and blood hemoglobin concentrations between 10 and 11 g/dL. Phosphate binder type and dosage were adjusted to maintain serum phosphate concentrations between 3.0 and 5.5 mg/dL per KDOQI recommendations.

Statistical Analysis

Descriptive statistics are reported as the mean (± SD) unless otherwise stated. Baseline characteristics including demographic and blood chemistry measures were compared between the treatment and placebo groups by one-way analyses of variance

(ANOVA). Pre- and post-intervention measurements within groups and between groups were compared using repeated measures ANOVA. Pre- and post-intervention biochemical and performance measurements were analyzed using one-way repeated measures analysis of variance (ANCOVA). Pearson correlation coefficients were calculated to examine associations between serum 25(OH)D and continuous variables at baseline and at 12 weeks. Multivariate regression was performed with change in the physical performance as the dependent variable while correcting for age, stride length, and the number of comorbidities to determine impact of 25(OH)D on performance in the 20-foot walk, TUG and RCS tests. Results were considered significant when $p<0.05$. Analyses were performed using SPSS 20.0 (IBM SPSS, Armonk, NY).

Results

A review of the medical history of 149 patients yielded 52 patients who met inclusionary criteria and agreed to participate (Figure 3.1). Patients were randomly assigned to either the placebo or the treatment group (n=26 for each group). Four patients did not complete the study – two voluntarily chose to withdraw; two had deteriorating health conditions independent of the study and two developed wounds, and thus no longer met study inclusionary criteria. Consequently, 25 patients completed the study in the placebo group and 21 in the treatment group.

Descriptive information on the participants by treatment group is presented in Table 3.1. The groups did not differ significantly in gender, age, race, years of HD treatment, BMI, Kt/V, or the incidence and number of comorbidities present. Further, the average step count taken over 3 days to provide a measure of baseline activity, did not differ significantly between the two groups (2019 steps for the placebo group vs 2383

steps for the treatment group). The groups were representative of the HD population of the three clinics regarding gender, age, and race. Supplements were administered during HD treatment under the direct observation of the site Registered Dietitian (RD) to confirm subject compliance.

Biochemistry Measurements

The groups did not differ significantly at baseline in serum concentrations of 25(OH)D, albumin, PTH, hemoglobin, corrected calcium, and phosphorus (Table 3.2). During the 12 week study, the mean serum concentration of 25(OH)D increased significantly in the group receiving the vitamin D supplement ($p<0.001$) and all participants achieved serum concentrations > 30 ng/mL. There was no significant change in the serum 25(OH)D concentration of the placebo group over the 12 week intervention period, and the serum 25(OH)D concentrations of all participants remained below 30 ng/mL (See Figures 3.2 and 3.3).

Plasma PTH concentrations decreased significantly in the vitamin D supplemented group (within group $p=0.02$ and between group $p<0.01$). Serum corrected calcium concentrations increased significantly in the vitamin D group (within group $p<0.01$ and between groups $p=0.04$); however, the mean serum concentrations were within the normal range for corrected calcium for both groups, both pre- and post-intervention. There were no differences within or between groups in the serum concentrations of albumin, hemoglobin, corrected calcium, or phosphorus at the completion of the intervention period and these concentrations were within acceptable target ranges.

Physical Performance Measurements and SF-36 Responses

There were no significant differences between the two groups in the timed 20-foot walk, TUG, or RCS at baseline or the end of the study (Table 3.3). However, there was a significant within group decrease in the RCS time for the supplement group from the beginning to the end of the intervention (19.7 ± 7.1 seconds vs. 17.0 ± 4.5 seconds, respectively; p=0.02) (Table 3.3 and Figure 3.4).

The best predictors of performance on the timed 20-foot walk, TUG, or RCS tests included stride length, age, and the number of comorbidities (see Table 3.4 for Pearson correlations). Stride length was positively correlated to physical performance while age and number of comorbidities were negatively correlated. When multivariate analysis was used to correct for age, stride length, and the number of comorbidities, serum 25(OH)D concentrations were not significant predictors of performance on the 20-foot walk, TUG, or RCS tests (Table 3.5).

There were no significant differences between groups on any of the SF-36 scores (Table 3.3). There was a positive correlation between serum 25(OH)D concentrations and most of the SF-36 categories including: physical functioning; physical health limitations; energy; emotional wellbeing; social functioning; pain; and general health (Table 3.6). However, with the exception of the physical health limitations and the emotional health limitations, the scores in these categories were correlated with the baseline serum 25(OH)D concentrations rather than the post-intervention 25(OH)D concentrations.

Discussion

This 12-week randomized, controlled trial providing vitamin D supplements to HD patients resulted in significantly increased serum concentrations of 25(OH)D and corrected calcium while significantly decreasing plasma concentrations of PTH when compared to the placebo group. The increased serum 25(OH)D concentrations in the intervention group were expected; the repletion protocol used in this study was consistent in regard to both dosage and time to see an effect with that used by other researchers to effectively increase serum 25(OH)D concentrations in patients with ESRD.[20-24]

The decreased plasma concentrations of PTH in the supplemented group versus the placebo was confounded by paricalcitol (a $1,25(OH)_2D$ analog) administration. Paricalcitol was, also, administered monthly based on plasma PTH, serum calcium, and serum phosphorus concentrations to all study participants to achieve serum PTH concentrations between 150 and 300 pg/mL.

Significant positive correlations were found between pre-intervention serum 25(OH)D concentrations and scores on the SF36 questionnaire. That is, individuals with higher serum 25(OH)D concentrations subjectively evaluated their general health and well-being higher than those individuals with lower serum 25(OH)D concentrations; however, increases in serum 25(OH)D due to supplementation did not significantly improve scores from baseline. This is consistent with the results from other studies.[21,25]

The performance on the 20-foot walk, TUG, and RCS did not significantly improve with the vitamin D supplementation and corresponding increased serum 25(OH)D concentrations. Several studies in individuals with normal renal function demonstrated improved physical function with vitamin D supplementation.[12-14] Pfeifer et

al.[13] found decreased body sway and decreased risk of falls in previously vitamin D deficient women after supplementation with vitamin D and calcium for 8 weeks. Likewise, Bunout et al.[12] found improved quadriceps muscle strength and faster gait in older adults after 9 months treated with vitamin D and calcium compared to individuals receiving a placebo. Although Dhesi et al.[14] did not see improvement in quadriceps muscle strength, they did find functional performance, reaction time, and balance were improved in older adults receiving vitamin D supplementation for 6 months.

Several reasons may account for why vitamin D supplementation did not significantly and positively impact physical performance in this study. The first possibility is that the 12-week study duration was insufficient for muscles to respond to the increased serum 25(OH)D concentrations. While Pfeifer et al.[13] saw improvement in physical performance in 8 weeks, the baseline physical activity of the study population was likely much greater than this study. Pfeifer's study was performed in Germany where the percentage of the population walking more than 30 minutes a day is three-fold higher than in the US (28.4% vs. 8.6%).[26] In addition, more than 50% of the participants in the Pfeifer study reported daily or weekly physical activity. In contrast, this study population averaged approximately 2,000 steps per day, well below the US average of approximately 5,100 steps per day and the recommended 10,000 steps per day for general health.[27] Bunout et al.[12] also showed that vitamin D supplementation paired with resistance training improved physical performance more than vitamin D alone over 9 months. It is possible that greater daily activity increases the efficacy of the vitamin D supplementation in improving muscle function by increasing the physical stimulus to increase gene transcription and myocyte differentiation and proliferation.

Due to the poor physical functioning of the study participants, participants were allowed to use the arms of the chair for assistance in performing the RCS test. Thus, the change in times pre- to post-intervention could have been the result of differences in assistance provided by arm chair use rather than true changes in physical performance secondary to vitamin D supplementation. Alternatively, it is possible that participants receiving the supplement used less assistance at the end of the intervention than at the beginning and that this improvement was not recognized as time was the outcome measure on this test.

Strengths and Limitations

This study was designed to and significantly improved the vitamin D status of HD patients, who began this study with serum 25(OH)D concentrations below 30 ng/mL. The dose and frequency of supplementation were consistent with repletion protocols for individuals deficient in vitamin D.[28] Moreover, supplements were provided during HD treatment which allowed for full compliance with consumption by participants.

This study was limited to testing participants at the HD clinics on the day of their HD treatment due to cost and transportation constraints. Thus, the physical performance measurements that could be collected were limited. Expanding this protocol to include the time to climb stairs or measure force or maximum lift capability could add useful data. In addition, although the study population was predominantly African American and representative of the racial distribution in the three clinics from where the participants came, the findings may not be generalized to all individuals on HD.

Conclusions and Recommendations

HD patients, deficient in vitamin D, who were supplemented with 50,000 IU vitamin D weekly for 8 weeks followed by 50,000 IU every two weeks for a duration of 12 weeks, showed improvements in serum 25(OH)D concentrations and plasma PTH concentrations. Further studies should include a larger sample size, a longer study duration (6 months or longer), and additional physical performance measurements. The study findings indicate that replenishment of deficient or insufficient levels of serum 25(OH)D concentrations in HD patients can be accomplished with vitamin D supplementation.

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Table 3.1: Group Characteristics at Baseline

	Placebo n=25	Treatment n=21	p
Gender			
Male/Female	14/11	10/11	0.58
Age (yrs)	59.8 ± 12.1	56.3 ± 12.4	0.35
Race			0.35
Caucasian	3	0	
African American	22	21	
Years on HD	4.9 ± 4.4	4.9 ± 3.6	0.97
Dialysis Location			
Langdale	10	10	
Auburn	9	9	
Tuskegee	6	4	
Average steps per day	2019 ± 1693	2383 ± 2909	0.61
Body Mass Index (kg/m^2)	29.8 ± 7.2	29.4 ± 5.8	0.83
Kt/V	1.7 ± 0.5	1.8 ± 0.5	0.49
Comorbidities			
Diabetes Mellitus	13	14	0.55
Hypertension	18	14	0.63
Cardiovascular Disease	14	10	0.16
Average #	2.7 ± 1.3	2.6 ± 1.2	0.76
Albumin (g/dL)	4.1 ± 0.3	4.1 ± 0.2	0.52
iPTH (pg/mL)	406.9 ± 296.7	435.2 ± 374.3	0.93
Hemoglobin (g/dL)	11.2 ± 1.4	11.4 ± 1.8	0.53
cCalcium (mg/dL)	9.5 ± 0.7	9.4 ± 1.0	0.28
Phosphorus (mg/dL)	5.4 ± 1.4	4.8 ± 1.4	0.52

Abbreviations are: cCalcium, corrected calcium. Values for age, years on HD, BMI, Kt/V are means ±SD. Values for gender, race, dialysis location and comorbidities are counts.

Table 3.2: Baseline and Final Measurements of Biochemical Markers by Treatment Group

Characteristics	Placebo (n=25)			Cholecalciferol (n=21)			
	Baseline	12 wk	Within Group P	Baseline	12 wk	Within Group P	Between Groups P
25(OH)D (ng/mL)	14.8 ± 5.8	16.0 ± 5.2	0.49	15.1 ± 4.8	51.8 ± 12.9	< 0.001	< 0.001
25(OH)D [n (%)]							
< 10 ng/mL	7 (28)	4 (16)		5 (22)	0 (0)		
10 to 20 ng/mL	15 (60)	14 (56)		15 (65)	0 (0)		
20 to 30 ng/mL	3 (12)	7 (28)		3 (13)	0 (0)		
> 30 ng/mL	0 (0)	0 (0)		0 (0)	23 (100)		
Albumin (g/dL)	4.1 ± 0.3	4.1 ± 0.3	0.21	4.1 ± 0.3	4.1 ± 0.2	0.73	0.42
iPTH (pg/mL)	406.9 ± 296.7	491.5 ± 587.0	0.22	420.2 ± 386.0	251.7 ± 264.8	0.02	<0.01
Hemoglobin (g/dL)	11.2 ± 1.4	11.5 ± 1.2	0.14	11.4 ± 1.9	11.7 ± 2.0	0.21	0.17
cCalcium (mg/dL)	9.5 ± 0.7	9.7 ± 0.5	0.09	9.4 ± 0.9	9.8± 0.9	<0.01	0.04
Phosphorus (mg/dL)	5.4 ± 1.4	5.5 ± 1.4	0.64	4.6 ± 1.4	5.1± 1.2	0.14	0.27

Abbreviations are: 25(OH)D, 25-hydroxyvitamin D; iPTH, intact Parathyroid hormone; cCalcium, corrected calcium. Values are means ± standard deviations. Numbers in parentheses are percentages.

Table 3.3: Baseline and Final Measures of Physical Measurements and SF-36 Responses by Treatment

Characteristics	Placebo (n=25)			Cholecalciferol (n=21)			
	Baseline	12 wk	Within Group P	Baseline	12 wk	Within Group P	Between Groups P
Timed 20 ft walk (seconds)	7.4 ± 2.2	7.4 ± 1.9	0.85	7.2 ± 2.2	7.6 ± 2.6	0.20	0.36
TUG (seconds)	12.8 ± 3.6	12.9 ± 4.2	0.93	13.1 ± 3.6	12.6 ± 3.4	0.35	0.67
Timed RCS (seconds)	18.0 ± 7.2	17.4 ± 6.3	0.58	19.7 ± 7.1	17.0 ± 4.5	0.02	0.34
SF-36 Scores							
Physical Functioning	66 ± 21	66 ± 22	0.84	62 ± 21	59 ± 27	0.35	0.74
Physical Health Limits	45 ± 35	51 ± 38	0.50	60 ± 39	56 ± 45	0.71	0.20
Emotional Health Limits	64 ± 42	78 ± 39	0.08	83 ± 23	75 ± 39	0.33	0.08
Energy	62 ± 22	64 ± 17	0.58	57 ± 17	55 ± 18	0.70	0.53
Emotional Well Being	82 ± 14	87 ± 14	0.16	78 ± 16	79 ± 18	0.79	0.10
Social Functioning	77 ± 19	84 ± 14	0.03	83 ± 19	76 ± 22	0.09	0.07
Pain	75 ± 26	71 ± 23	0.42	69 ± 24	59± 24	0.09	0.96
General Health	49 ± 19	48 ± 20	0.85	47 ± 20	50± 23	0.47	0.59

Abbreviations are: JUG, jump up and go test; RCS, repeated chair stand; SF-36, Medical Outcomes Study 36 Item Short Form Health Status Questionnaire. Values are means ± standard deviations.

Table 3.4: Pearson Correlations related to Physical Performance

	Post 20 ft walk	Pre-JUG	Post-JUG	Pre-RCS	Post-RCS	Stride length	Avg steps per day	Age	Pre-Kt/V	Pre-P	Number Comorb.
Pre25,OHD		-0.31*							0.30*		-0.32*
Post25,OHD						0.34*	0.32*				
Pre20 ft walk	0.82**	0.88**	0.81**	0.89**	0.68**	-0.84**	-0.36*	0.55**		-0.41**	0.49**
Post20 ft walk		0.76**	0.78**	0.67**	0.68**	-0.72**		0.56**		-0.46**	0.46**
PreJUG			0.81**	0.90**	0.67**	-0.74**		0.57**		-0.42**	0.57**
PostJUG				0.73**	0.86**	-0.70**		0.61**		-0.39**	0.40**
PreRCS					0.69**	-0.72**		0.50**		-0.36**	0.51**
PostRCS						-0.59**		0.57**		-0.31*	0.36*
Stride length							0.37*	-0.47**		0.33*	-0.35*
Avg steps per day								-0.49**	0.31*		

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Abbreviations: JUG – jump up and go test; RCS – repeated chair stand; Number Comorb. – average number of comorbidities per participant, Pre25,OHD – pre intervention 25,hydroxyvitamin D level.

Table 3.5: Multivariate Models of Performance Measures adjusted for age, stride length and number of comorbidities

		Adj. R^2	β	CI 95%		P
Multivariate Models				Lower	Upper	
Timed 20 Ft Walk						
	Pre 25(OH)D	0.70	-0.03	-0.10	0.07	0.70
	Post 25(OH)D	0.72	0.15	0.00	0.03	0.08
Timed JUG						
	Pre 25(OH)D	0.63	-0.05	-0.25	0.15	0.63
	Post 25(OH)D	0.67	0.06	-0.03	0.05	0.54
Timed RCS						
	Pre 25(OH)D	0.52	-0.05	-0.44	0.28	0.66
	Post 25(OH)D	0.54	0.03	-0.05	0.07	0.80

Table 3.6: Pearson Correlations related to SF-36 Scores

	Pre Physical Fun	Pre Physical Health Limit	Pre Emotion Heath Lmt	Pre Energy	Post Energy	Post EmWB	Pre Social Fun	Post Social Fun	Pre Pain	Pre General Health	Post General Health
Pre25(OH)D	0.30*			0.36*	0.41**	0.33*	0.49**	0.36*	0.32*	0.39**	0.44**
Post25(OH)D		0.34*	0.35*								

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Abbreviations: Pre/Post25OHD, pre and post intervention levels of 25,hydroxyvitamin D; Physical Fun, physical functioning section of SF-36 questionnaire; EmWB, Emotional Well Being;

Figure 3.1: Flow diagram of subject recruitment

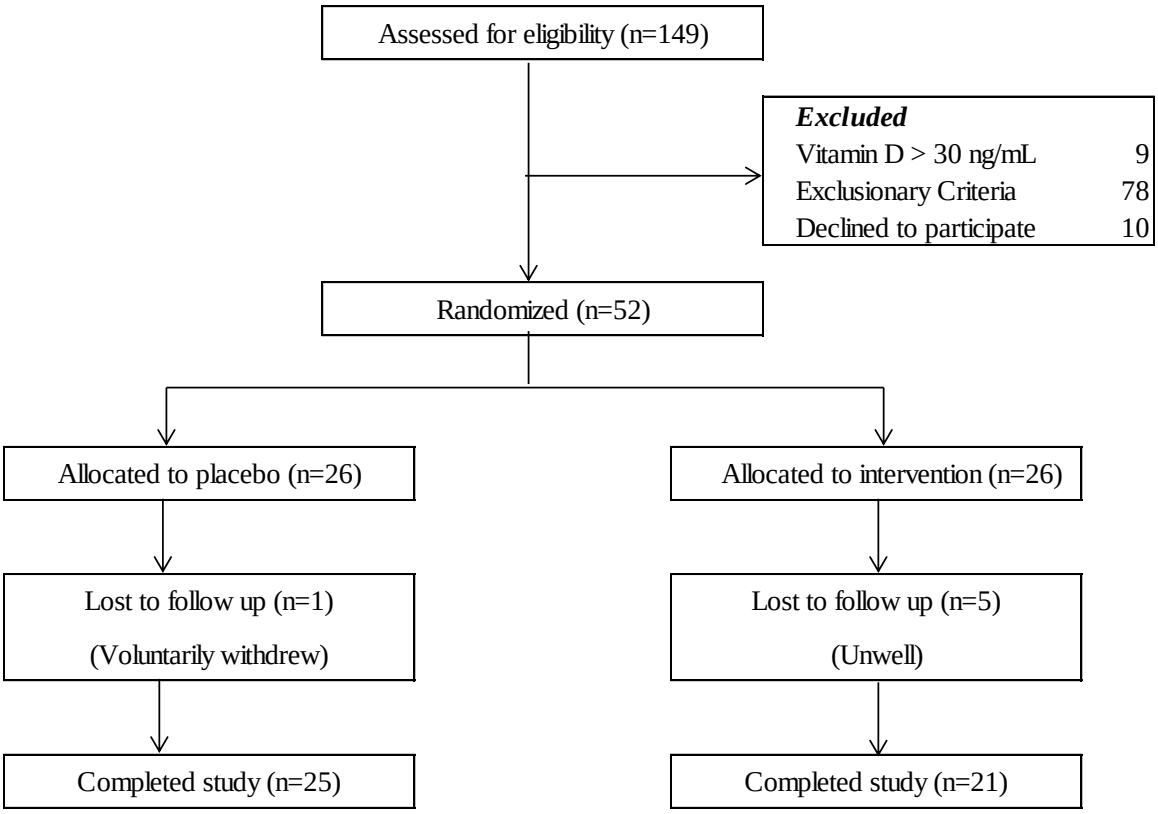


Figure 3.2

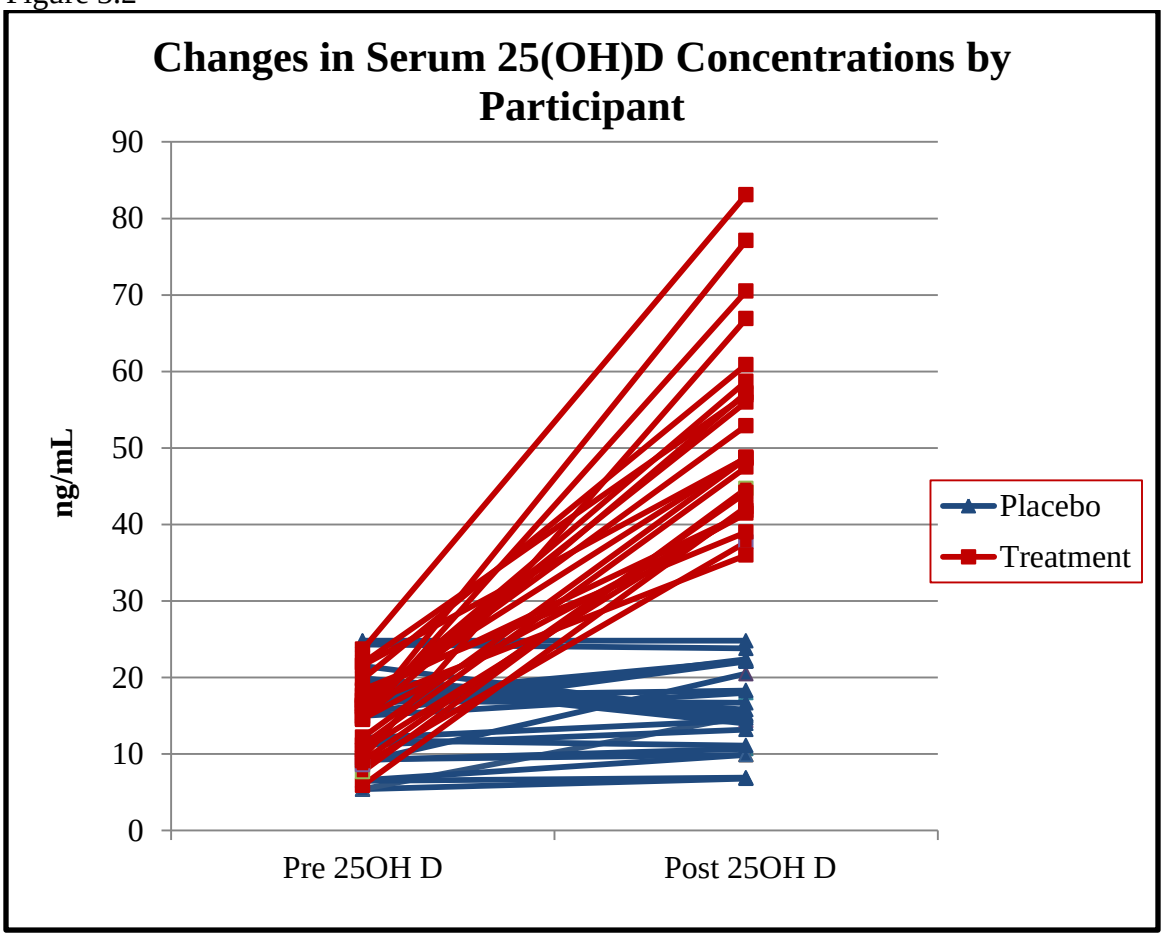

Changes in Serum 25(OH)D Concentrations by Participant
ng/mL
90
80
70
60
50
40
30
20
10
0
Pre 25OH D
Post 25OH D
Placebo
Treatment

Figure 3.3: Mean Serum 25(OH)D Concentrations Pre- and Post-Intervention by Treatment

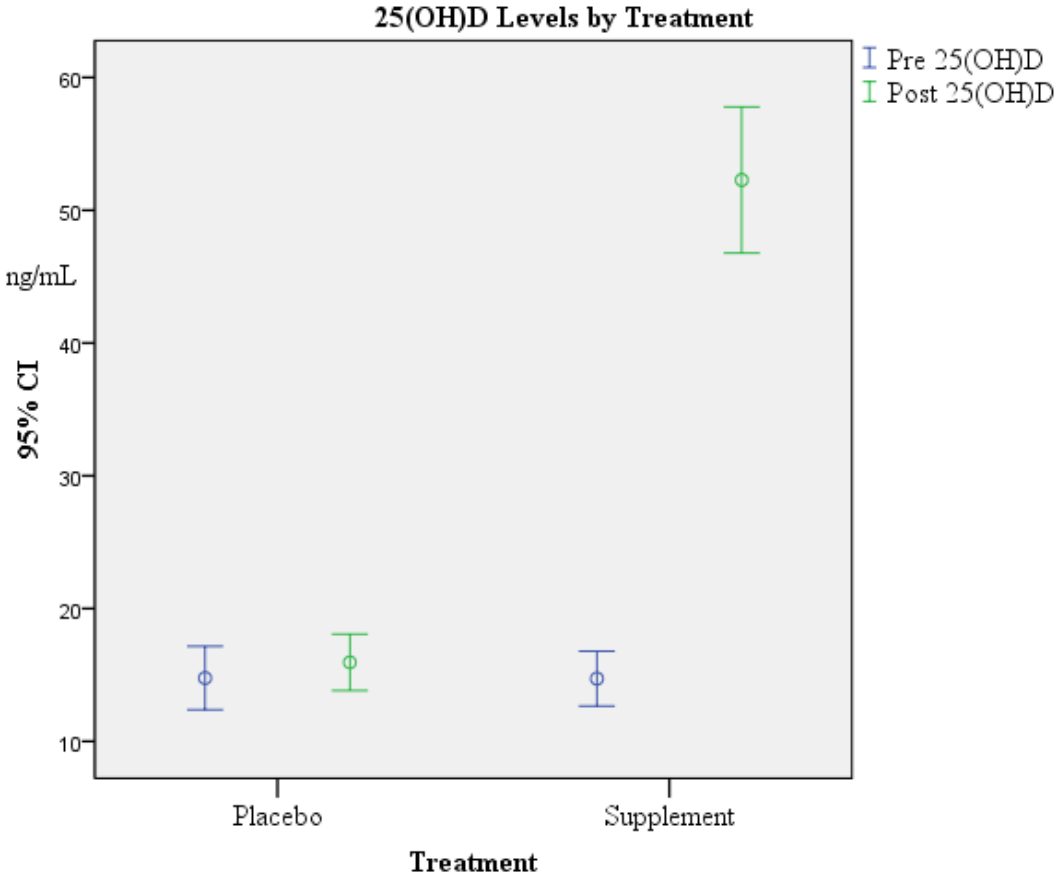
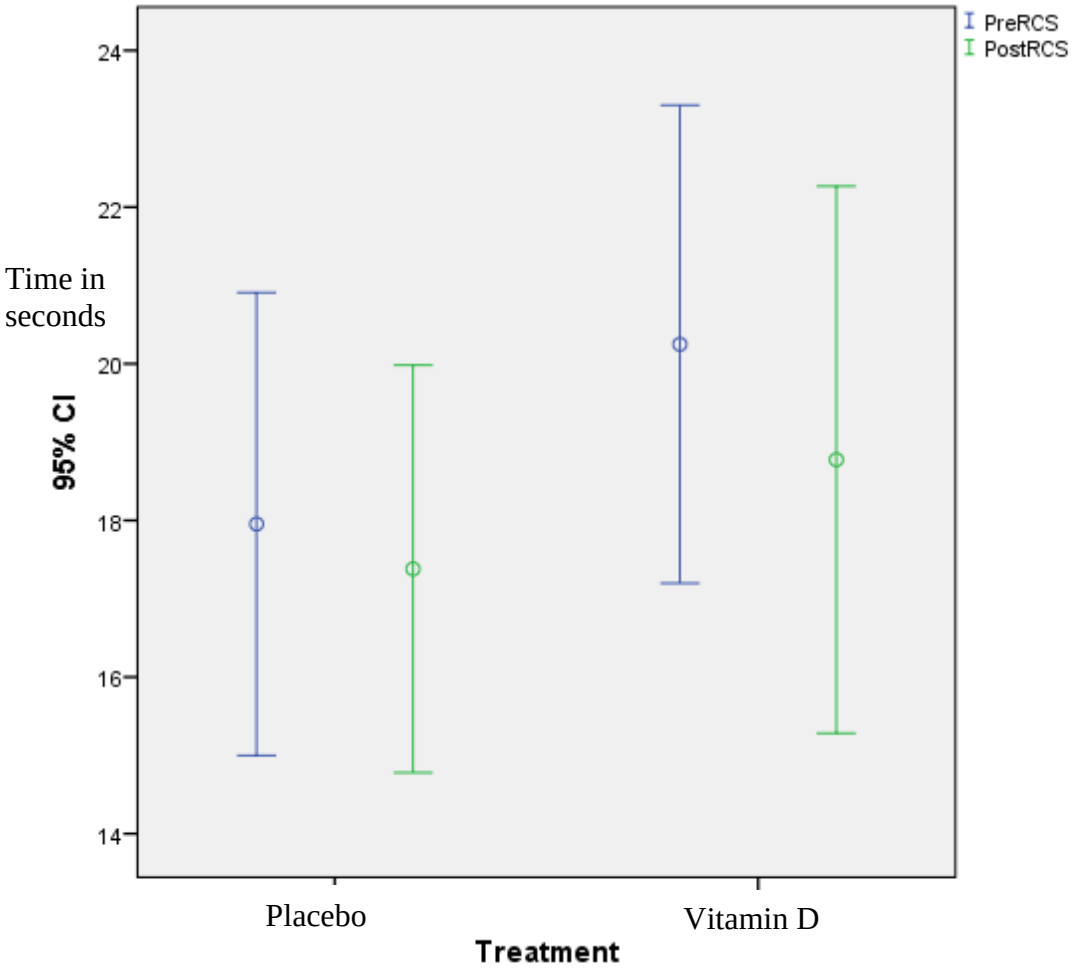


Figure 3.4: Mean RCS Times Pre- and Post-Intervention by Treatment



Chapter 4

Summary of Findings

1. There was a significant increase in serum 25(OH)D concentrations of the treatment participants from baseline to completion of the 12 week intervention. This finding supports the first hypothesis
2. There was no significant difference in serum 25(OH)D concentrations of the placebo group from baseline to completion of the 12 week intervention. This finding supports the second hypothesis.
3. There was a significant decrease in plasma PTH and increase in serum corrected calcium concentrations within the treatment group and between groups. This does not support the third hypothesis. There was no significant difference between groups in serum albumin, hemoglobin or phosphorus concentrations. This does support the third hypothesis.
4. There was no significant correlation between serum concentrations of 25(OH)D and performance of a timed 20 foot walk. This does not support the fourth hypothesis.
5. In a multivariate analysis correcting for age, stride length and number of comorbidities, increased serum 25(OH)D concentrations approached significance in predicting performance on a timed 20 foot walk. This partially supports the fifth hypothesis.

6. There was a significant correlation between serum concentrations of 25(OH)D and performance of a timed JUG test before the intervention. This supports the sixth hypothesis.
7. Increased serum concentrations of 25(OH)D did not predict improvement in the performance of a timed JUG test. This did not support the seventh hypothesis.
8. There was no significant correlation between serum concentrations of 25(OH)D and performance of a timed RCS test. This did not support the eighth hypothesis.
9. Increased serum concentrations of 25(OH)D in the treatment group did not predict improvement in the performance of a timed RCS test. This did not support the ninth hypothesis.
10. There was significant correlation between serum concentrations of 25(OH)D and scores on the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire. This supports the tenth hypothesis.
11. Increased serum concentrations of 25(OH)D did not predict improvement in scores on the Medical Outcomes Study 36 Item Short Form Health Status Questionnaire. This did not support the eleventh hypothesis.

Chapter 5

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Appendices

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Subject:	Protocol approved, #11-208 MR 1107
From:	Human Subjects (HSUBJEC@auburn.edu)
To:	awj0002@tigermail.auburn.edu;
Cc:	groppss@auburn.edu; oneilm1@auburn.edu;
Date:	Thursday, November 10, 2011 11:41 AM

Dear Ms. Johnson,
As you know, your protocol entitled "The Effect of 25, OH Vitamin D Supplementation on Physical Performance and Muscle Mass in Ambulatory Patients on Hemodialysis " received final approval as "Minimum Risk " under federal regulation 45 CFR 46.
Official notice:
This e-mail serves as official notice that your protocol has been approved. A formal approval letter will not be sent unless you notify us that you need one. By accepting this approval, you also accept your responsibilities associated with this approval. Details of your responsibilities are attached. Please print and retain.
Consent document:
Since you already have in your possession the approved, stamped consent and flyer, please conduct your study at your convenience. You must use copies of the consent document when you consent participants, and provide a copy (signed or unsigned) for them to keep.
Expiration:
Your protocol will expire on July 11, 2012. Put that date on your calendar now. About three weeks before that time you will need to submit a final report or renewal request. (You might send yourself a delayed e-mail reminder for next June.)
If you have any questions, please let us know.
Best wishes for success with your research!

Susan

Susan Anderson, IRB Administrator
IRB / Office of Research Compliance
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