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EFFECTS OF VITAMIN D SUPPLEMENTATION ON MOTOR SYMPTOMS OF PATIENTS WITH PARKINSON'S DISEASE

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B.S., University of Georgia, 2007

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ABSTRACT

EFFECTS OF VITAMIN D SUPPLEMENTATION ON MOTOR SYMPTOMS OF PATIENTS WITH PARKINSON'S DISEASE

By Stephanie DuBose

Parkinson's disease (PD) is the second most common neurodegenerative disorder and it disproportionally affects older populations. There is biological plausibility for the hypothesis that vitamin D may offer neuroprotective benefit for patients with PD or could improve clinical PD symptoms. This study aims to examine whether high dose vitamin D supplementation, over the Recommended Daily Allowance (RDA), has a significant effect on motor symptoms in patients with PD. A randomized, active placebo-controlled, double-blind, parallel group pilot clinical trial was conducted to evaluate the association of oral Vitamin D supplementation with PD-related motor symptoms. Thirty patients with PD and low levels of serum 25-hydroxyvitamin D [25(OH)D $\leq$ 30 ng/ml] were randomly assigned to either high dose vitamin D supplement (50,000 IU weekly dose plus 600 IU/day) or to low dose supplement (weekly placebo plus 600 IU/day). Three motor outcomes of primary interest were assessed at baseline and after 3 and 6 months of treatment with vitamin D supplement. When mean scores for these motor assessments were plotted over time, there were no consistent patterns of improvement in the treatment group as compared to the active placebo group for most outcomes. However, the mean time to complete the Timed-Up-and-Go (TUG) during the "off" medication state decreased over time for the treatment group (mean change = -1.4 seconds), but increased for the placebo group (mean change = 2.7 seconds). Despite the diverging trends for this motor test, results from repeated measures ANOVA analysis indicated no statistically significant improvement for the treatment group over placebo group for any of the motor outcomes over time. These preliminary results suggest that vitamin D supplementation had minimal impact on PD-related motor symptoms.

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BACKGROUND

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and, like Alzheimer's, it disproportionally affects older individuals. Parkinsonism is the typical clinical phenotype of PD, and is characterized by resting tremor, bradykinesia (abnormally slow movement), cogwheeling limb rigidity, gait disturbance (slowed, shortened steps), and postural instability (1, 2). The pathological hallmark of PD is a pronounced loss of dopamine-producing neurons in the substantia nigra pars compacta, located in the midbrain. This loss results in a drastic depletion of dopamine in the striatum, a central component of the basal ganglia that is situated at the base of the forebrain and is responsible for both the initiation and control of movement (1, 3). Over time, PD may lead to severe incapacity and can seriously impair an individual's quality of life.

There are two forms of PD—familial and sporadic, with approximately 90% of PD cases thought to be sporadic. The exact pathogenetic mechanisms underlying the selective dopaminergic cell loss in PD are still not understood. Current thinking is that a combination of mitochondrial dysfunction, oxidative stress, inflammation, and protein mishandling have a central role in PD pathogenesis, and that in sporadic PD these processes are probably induced by non-genetic factors interacting with susceptibility genes (4).

Epidemiology and Risk Factors of Parkinson's Disease

The prevalence of PD in industrialized countries is generally estimated at 0.3% of the entire population (4). Disease prevalence is age-associated, with approximately 1% of the worldwide population being affected at age 65 years, increasing to 4–5% by 85 years (1). The median age of onset for all parkinsonian syndromes is 61.6 years. Onset before age 30 is rare, but 5-10% of PD cases begin by age 40 (5). More males are affected than females (in a ratio of 1.5:1.0), but whether this reflects workplace exposure, sex-linked genetic variability, a protective effect of estrogen, or some other factor(s) is unknown (1). Some studies in the U.S. have indicated that PD might be less prevalent in black and Asian persons than in white persons, but results are conflicting and reported differences may result from differences in response rates, survival, and case-ascertainment rather than from real differences in PD prevalence across ethnic groups (4). Incidence rates of PD appear to be comparable worldwide.

Several risk factors, including environmental toxins, have been associated with the development of PD. The most compelling evidence for an environmental factor in PD relates to the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (2, 6). In 1983, a group of intravenous drug users unwittingly injected drugs contaminated with MPTP and quickly developed an acute, permanent parkinsonian state (6). The subsequent finding that MPTP selectively damages dopaminergic cells in the substantia nigra led to the hypothesis that exposure to environmental toxins might be related to the risk of PD (4). Although MPTP exposure is extremely rare and does not likely contribute to the number of incident cases, the MPTP model has become one of the most extensively studied models of PD.

Some epidemiological studies indicate that rural living, pesticide use, well-water consumption and certain occupations, including mining and welding, are associated with an increased risk of PD, but these studies are subject to methodological limitations (4). Evidence fairly consistently points toward a positive association between pesticide exposure and PD risk (4). Exposure to a combination of maneb and paraquat has been associated with increased PD risk (7). However, due to challenges in exposure assessment, there is minimal human data to support the association of PD with specific pesticides. Inverse associations have been observed for cigarette smoking, caffeine, and alcohol intake, although the mechanisms for how these agents might protect against PD are not fully understood (1). For caffeine, the inverse association appears to be stronger among men than women. This may be related to an interaction between caffeine and estrogen (4). Findings regarding the association between alcohol and PD have been less consistent (4).

Several causative monogenetic mutations have been identified in PD, including α-synuclein and parkin (3). Approximately 5–10% of PD patients have a familial form of parkinsonism with an autosomal-dominant pattern of inheritance (2). Large pedigrees have been identified where members in different generations suffer from PD. In addition, the incidence of PD is greater in family members than in age-matched controls (2). The Tanner et al. twin study reported no difference in concordance between monozygotic and dizygotic twins of PD patients aged 60 years or older but a significantly increased incidence was observed in monozygotic twins of PD patients who developed PD at less than 50 years of age (8). This suggests that genetic factors are important in young-onset patients but are not as likely to play a major role in patients with sporadic PD (2).

Role of Vitamin D in Parkinson's Disease

It has been hypothesized that insufficient vitamin D may play a role in the pathogenesis of PD. Vitamin D (calciferol) comprises a group of fat soluble secosterols that are found in very few foods naturally (9). Vitamin D comes in two major forms—vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Both vitamin D_2 and vitamin D_3 appear to be fully active in humans (9). Vitamin D_2 originates from yeast and a plant sterol, ergosterol; vitamin D_3 originates from 7-dehydrocholesterol, a precursor of cholesterol, and is synthesized in the skin with sun (ultraviolet B, UV-B) exposure (9). Because vitamin D can be synthesized in the human body and has actions that affect other physiological processes within the body, it is no longer considered a vitamin, but rather a hormone.

Vitamin D from the diet or sunlight exposure is rapidly transported to the liver for storage. A specific liver 25-hydroxylase enzyme slowly releases 25-hydroxyvitamin D [25(OH)D] into the blood to achieve a concentration related to the total liver storage (9). This, coupled with a relatively long half life of 10 days to 3 weeks (9), makes serum 25(OH)D a reliable indicator of individual human vitamin D status. Major determinants of 25(OH)D concentration include latitude (or more specifically, exposure to UV-B radiation), season, age, skin tone, and body mass index (BMI). In the United States, dietary and multivitamin or calcium/vitamin D supplement intake provide a minimal contribution to circulating levels (10).

Vitamin D and the circulating 25(OH)D are biologically inactive at physiological concentrations (9). Circulating 25(OH)D is converted by kidney 1-alpha-hydroxylase

(1-α-OHase) to the biologically active calcitriol, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$], which has a short serum half life of 4 to 6 hours, and regulates calcium absorption, bone metabolism, and serum calcium concentrations (9). This renal production of 1,25-$(OH)_2D_3$ is tightly regulated by parathyroid hormone and calcium and phosphorus levels (11). In the last decade, several other cell types have also been demonstrated to express 1-α-OHase and to activate circulating 25(OH)D to the biologically active calcitriol (9). It was previously assumed that the brain 1,25-$(OH)_2D_3$ supply was dependent on the serum or plasma concentration of 1,25-$(OH)_2D_3$ (12). However, recent data demonstrating the brain localization of 25-hydroxylase and 1-α-OHase enzymes suggest that the central nervous system (CNS) can locally perform the bioactivation of the vitamin D_3 prohormone (12). Further, the nuclear functions of 1,25-$(OH)_2D_3$ are mediated through the vitamin D receptor (VDR) and evidence has accumulated to suggest that both mRNA encoding the VDR and the protein itself are present in the nervous system (12, 13).

Because vitamin D regulates a vast range of physiological processes that go amiss in disease states, including cell proliferation, differentiation, and survival, as well as resistance to oxidative stress, regulation of other hormones, and immune modulation, it is not surprising that insufficient or low vitamin D has been associated with a variety of clinical disorders and chronic diseases (10). Low vitamin D has been associated with impaired balance and decreased muscle strength in community dwelling, ambulatory individuals (14), mood and cognitive dysfunction, autoimmune disorders such as multiple sclerosis and diabetes mellitus, and certain forms of cancer. As noted above, it has recently been proposed that low vitamin D levels may play a role in the pathogenesis or

progression of PD (9). Both 1-α-OHase and VDR are widely distributed in areas of the brain known to be affected in disorders of gait and balance. Importantly, both 1-α-OHase and VDR are most heavily concentrated in the substantia nigra, the area of the brain preferentially affected in PD. Also, vitamin D is involved in regulation of tyrosine hydroxylase gene expression, which can regulate dopamine biosynthesis, and in the expression of brain-derived neurotrophic factor (BDNF) and glutathione, both of which are implicated as possible neuroprotective compounds in PD (15).

There is biological plausibility backing the hypothesis that vitamin D may offer neuroprotective benefit for patients with PD or improvement for clinical PD symptoms. Neurotoxin models of PD generally indicate that vitamin D may offer neuroprotection for dopaminergic neurons (10). Similar to many pharmacologic phenomena, these neuroprotective effects exhibit a U-shaped curve, with loss of neuroprotection or even detriment at higher, presumably supraphysiological doses (10). Furthermore, vitamin D may also be a necessary cofactor or augment the neuroprotective effects of other compounds such as progesterone (16). Gestational vitamin D deficiency appears to have deleterious effects in newborn and adult rodents and supplemental vitamin D improves dopaminergic survival in rodents (17). However, at this point, it is not clear whether vitamin D deficiency in mature animals leads to increased susceptibility to neurodegenerative disease or whether supplemental vitamin D administered after a toxic insult (but before morphological or behavioral sequela become apparent) could offer neuroprotection (10).

The association with and possible causal role of insufficient vitamin D in many chronic diseases is becoming more widely appreciated, yet what constitutes an optimal

blood concentration of vitamin D for humans, and specifically for the human nervous system, remains unknown (10). Older published studies typically use lower cutoffs for vitamin D deficiency (< 20 ng/ml), based on concentrations needed to avoid rickets and osteopenia. The Institute of Medicine (IOM) maintains that a serum 25(OH)D concentration of greater than or equal to 20 ng/ml is adequate (18). However, the cutoffs for defining vitamin D sufficiency in more recently published literature are higher. Insufficiency is typically defined as 25(OH)D < 30 ng/ml. Many vitamin D researchers suggest that 25(OH)D concentration should be above 30 ng/ml (19, 20). Other investigators have suggested that optimal levels are even higher, but data supporting this are lacking. There may be a U-shaped curve for the most advantageous levels, as the observational study by Melamed et al. found that there was a lower risk of mortality at concentrations of 30 to 49 ng/ml but that concentrations of greater than 50 ng/ml placed women at a higher risk of death (21). While ideal serum concentrations have yet to be determined, the IOM recently reviewed the literature on the possible health effects of vitamin D and updated the recommended dietary intake levels. The new recommendations are 600 IU per day for adults age 70 years and under and 800 IU per day for those 71 years and older (18).

Human PD studies of vitamin D status have consistently reported lower vitamin D concentrations in cases compared to control subjects (15, 22-24). One obvious explanation is that PD is a chronic disease, and as patients with PD become more severely affected, they have less UV-B exposure and are therefore less likely to get adequate intake of vitamin D. Consistent with this theory, Sato's studies suggest that the prevalence of vitamin D insufficiency correlates with disease duration and severity (24).

However, other studies do not support such an explanation. Evatt et al. found that significantly more patients with PD had insufficient vitamin D than both healthy controls and patients with Alzheimer's disease, another chronic, neurodegenerative disorder (22). An association between vitamin D concentrations and disease symptom duration could not be confirmed (22). In a subsequent analysis of stored serum from the DATATOP trial of patients with early PD and only mild symptoms not yet requiring medical treatment, Evatt et al. found a high prevalence of vitamin D insufficiency (69%) and deficiency (26%) (15). In this cohort, vitamin D levels did not decrease over the study period, but actually increased (15). These findings are consistent with the possibility that chronic insufficiency is present before the clinical manifestations of PD and may play a role in the pathogenesis or progression of PD. Additionally, the analysis of serum from the Mini-Finland study found that increasing quartile of 25(OH)D (measured decades before clinical diagnosis of PD) was associated with decreased risk of PD (23). The adjusted relative risk comparing the highest and lowest quartiles was 0.33 (95% CI 0.14-0.80) (23). Knekt et al. observed a correlation between 25(OH)D concentration and decreased PD incidence. It should be noted that mean 25(OH)D concentrations reported in the entire cohort (both cases and non-cases) were suboptimal (23). Thus, it remains unknown whether concentrations above 30 ng/ml would be associated with further PD risk reduction.

Further studies are necessary to help understand the potential role of vitamin D insufficiency in the pathogenesis or progression of PD. A pilot study was undertaken in an effort to provide preliminary data on dose-response and possible symptomatic benefit of vitamin D supplementation in patients with PD, to help guide future research.

Objectives of the study included determining the adequacy of two supplement strategies to improve vitamin D status in PD patients with vitamin D insufficiency and determining the impact of such strategies for both motor and non-motor symptoms in PD. If there appears to be no symptomatic benefit, future studies should focus on neuroprotection, considering whether vitamin D can help prevent the development of PD, or at least slow the progression. If, however, there appears to be symptomatic benefit, this would be critical in the design of treatment trials focusing on both short-term and long-term potential benefits of vitamin D.

This thesis is a preliminary analysis of the pilot study and aims to examine whether high dose vitamin D supplementation, over the Recommended Daily Allowance (RDA), has a significant effect on motor symptoms in patients with PD. The primary hypothesis is that correction of vitamin D deficiency will improve PD-related motor symptoms, thus improving and/or avoiding PD-related co-morbidities. Other exploratory topics of interest include whether there is a dose-response relationship between vitamin D concentrations and impact on motor symptoms and whether the effect of vitamin D supplementation on serum 25(OH)D concentration in patients with PD is different, as compared to the general population.

METHODS

Study Design

The Pilot Study of Vitamin D Repletion in Patients with Parkinson's Disease (ViDiP Pilot) is a randomized, active placebo-controlled, parallel group pilot clinical trial. The active placebo group was included because previous PD clinical trials have demonstrated significant placebo benefit that may last months (25). This study was approved by the Emory University Institutional Review Board.

Study Population and Setting

Participants were recruited from the patient population attending the Emory University Movement Disorders Clinic, were members of Atlanta Area PD support groups, or saw the study advertised in PD patient newsletters. Patients with PD who were age 18-89 years, in Hoehn and Yahr (H&Y) stages I-IV, and who had serum 25(OH)D concentration $\leq$ 30 ng/ml within the previous three months were eligible to enroll. Other eligibility requirements included being able to complete the study questionnaires and study measures, capable of informed consent, and free of active cancer or other serious medical conditions that might reasonably preclude their completing the 6 month intervention. Also, participants had to be able to complete an 8 meter walk at screening/baseline evaluation. Exclusion criteria included a history of hypercalcemia, hypercalciuria, liver failure, end-stage renal disease (National Kidney Foundation Classification Stage 5) or kidney stones within the past 5 years, concurrent infection (e.g.,

osteomyelitis) that may have interfered with study procedures, and a history of unresolved or measured glomerular filtration rate (GFR, estimated or measured within the previous year) <15 ml/min (25).

Clinical Trial Protocol

All age eligible patients with PD who had serum 25(OH)D concentration ≤ 30 ng/ml within the previous three months were identified as potential study participants and invited for a screening visit. Informed consent was obtained from individuals who met the above inclusion requirements and agreed to participate in the study (25). A study schema is provided in Figure 1.

After confirming that the participant met inclusion/exclusion criteria as detailed above, subjects were randomized per a block randomization schedule provided by the co-investigator (Dr. Vin Tangpricha, who did not meet or perform any clinical assessments on the participants). The code allowed study personnel to dispense treatment medication in double-blinded fashion after the Screening/Baseline Evaluation was complete (25).

Participants were randomized to treatment or active placebo group and received study medications and instructions. At the end of the screening/baseline visit, participants received a supply of study medications for either: a) **treatment** (High Dose Supplement-oral Vitamin D_3 50,000 IU capsule weekly for 26 weeks plus daily vitamin D supplement containing 600 IU vitamin D); or b) **active placebo** [Standard Dose Supplement-Placebo (indistinguishable from active vitamin D_3 capsules) weekly for 26 weeks plus daily vitamin D supplement containing 600 IU vitamin D]. With this dosing, all participants received a daily supplement with the RDA for vitamin D (600 IU daily). In addition, half

of the participants received 50,000 IU vitamin D weekly for 26 weeks; the other half of participants received placebo capsules for 26 weeks. Participants returned at 3 months (± 2 weeks) and 6 months (± 2 weeks) for follow-up (25).

Data Collection

Cognitive performance was evaluated at baseline using a Mini Mental State Examination (MMSE). Affective assessments administered at baseline were the Geriatric Depression Scale (GDS), Beck Depression Inventory (BDI), and Beck Anxiety Inventory (BAI).

Phlebotomy for 25(OH)D was performed at each visit (screening/baseline, 3 month, and 6 month), as well as the following motor assessments: Timed 8 Meter Walk (measured in seconds), TUG test - Timed "Up and Go" (measured in seconds), and Unified Parkinson's Disease Rating Scale (UPDRS) Part III (Motor Examination). Subjects completed two separate trials for the Timed 8 Meter Walk, which were later averaged. The latter two functional (motor) measures were done in the 'on' and 'off' medication states. The 'on' state refers to when the patient is having a good response to medication and minimal symptoms, whereas the 'off' state describes when medication is not working.

The **Timed 8 Meter Walk** is the time required to walk an 8 meter course, measured to the nearest tenth of a second. Walking aids may be used on this test, but the participant must be able to walk without assistance from another person. **TUG** is a screening tool that was developed to identify elderly patients at risk of falling, and the test is capable of distinguishing those who have balance problems from those who do not

(26). This test times the subject as they rise from a chair, walk 3 meters, turn around, return to the chair, and sit down. The test is performed twice, with the first being practice and the second, the actual test. The instructions for the test are as follows: “Start sitting in the chair with your back against the chair and your feet flat on the floor. Stand up, walk as quickly and as safely as you can, cross the tape, turn around, walk back to the chair, and sit down.” TUG scores have been found to correlate moderately well with gait speed ($r = -0.55$), scores on the Berg Balance Scale ($r = -0.72$), and the Barthel Index ($r = -0.51$) (27). **UPDRS** is commonly used by clinicians and researchers to measure the severity and progression of PD. Part III of the UPDRS, an outcome of primary interest in this study, assesses motor function.

All testing took place at Wesley Woods Outpatient Clinic. Marian Evatt, M.D. conducted the physical examinations and UPDRS evaluations. Elaine Sperin, ViDiP Study Coordinator, conducted the remainder of the evaluations. The Emory University Movement Disorders section lead coordinator, who has several years of experience administering these tests, trained Ms. Sperin on conduct of neuropsychological testing. A licensed clinical neuropsychologist, whose research focus is neurodegenerative disease, was available for assistance with scoring and questions. All of the testing for each visit was completed within a 4-6 hour epoch. Testing was completed in a room without any distractions, such as ringing phones, beepers, etc.

Serum 25(OH)D concentrations were analyzed in blinded fashion using an enzyme-linked immunosorbent assay kit for 25(OH)D (IDS, Inc., Fountain Hills, Arizona). The kit’s limit of detection is 2.0 ng/ml. Individual samples were run in duplicates and batches of 40 to minimize inter-assay variability. Quality assurance for

determination of 25(OH)D concentration was ensured by participation in the vitamin D external quality assessment scheme (DEQAS). The intra-assay and inter-assay coefficients of variation of the 25(OH)D enzyme-linked immunosorbent assay are less than 8.0% and less than 10.0%, respectively. Duplicate samples that had coefficients of variation greater than 10.0% were repeated.

Data Analysis

Exploratory analyses were performed to become familiar with variables in the data set and to search for missing and implausible values. Treatment groups were assessed for comparability of demographic characteristics at baseline using Fisher's exact test for categorical variables and ANOVA for continuous variables. Means and standard deviations were examined for each continuous variable and frequency distributions were examined for categorical variables, both overall and by treatment group.

The effect of vitamin D supplementation was evaluated by assessing the difference of each motor symptom outcome of interest from baseline to 6 month follow-up between patients in the treatment and placebo groups. Each outcome of interest was plotted over time for each individual. Mean values for each outcome of interest were also plotted over time for each group.

Repeated Measures ANOVA was used to assess the change in the outcome measures of interest over time, between the treatment and placebo groups. The SAS procedure (Proc) Mixed was utilized to fit a linear model with repeated measures. Using Proc Mixed, a separate model was run for each of the five dependent variables of

interest—Timed 8 Meter Walk, TUG during both the “on” and “off” states, and UPDRS III subscore during both the “on” and “off” states. The model included terms representing treatment group (treatment or placebo), visit (baseline, 3 months, or 6 months), and the interaction between treatment group and visit. Intent-to-treat analysis was performed; analyses were based on randomization treatment assignment, regardless of adherence.

To explore the topic of how each vitamin D supplementation dose affected circulating vitamin D concentrations, paired t-tests were performed on both treatment and placebo groups to evaluate whether mean serum 25(OH)D concentrations changed significantly over time. T-tests were also used to evaluate whether the mean serum 25(OH)D was statistically different between the two groups at all time points (baseline, 3 months and 6 months). To assess whether the effect of vitamin D supplementation may differ for patients with PD as compared to the general population, the mean overall change in each group’s serum concentration was compared to previous findings in the literature for healthy populations. The proportion of each group that reached an adequate serum concentration was also obtained.

To consider whether there was a dose-response relationship between vitamin D concentration and motor symptoms, a linear regression model was fit. The model assessed whether 25(OH)D concentration was associated with motor outcome variables of interest, regardless of treatment group. Proc Reg (SAS Institute, Cary, NC) was used to fit ten linear regression models—five unadjusted and five multivariate-adjusted for each of the motor outcomes of primary interest (Timed 8 Meter Walk, TUG during both the “on” and “off” states, and UPDRS III subscore during both the “on” and “off” states). The multivariate model adjusted for all potential confounders. These confounders were

decided upon, *a priori*, based on a directed acyclic graph (DAG) and biological plausibility relating each of the potential confounders to both motor outcomes and serum 25(OH)D concentration. Collinearity was assessed and was determined not to be an issue in these models. Proc Glm (SAS Institute, Cary, NC) was also used to calculate the least squares means for each motor outcome, across quartiles of serum 25(OH)D concentration. The unadjusted model was used to obtain these estimates.

All statistical analyses were conducted using SAS Version 9.2 software (SAS Institute Inc., Cary, NC). A cutoff level of $p < 0.05$ (two-sided) was used for assessing statistical significance.

RESULTS

Study Participants

Thirty-one participants were recruited for the pilot study; however, one was not eligible due to claudication. The total sample size was 30. Randomization resulted in 16 participants in the treatment group and 14 participants in the placebo group. See study flow diagram (Figure 2). Treatment groups did not differ significantly ($p > 0.05$) on characteristics measured at baseline (Tables 1 and 2), suggesting that randomization was successful. The mean age of participants in the study was 64 years. Sixty-three percent were men, 87% were white, and 31% were obese (BMI $\geq$ 30). Most participants had either some college education or were college or graduate school educated. Participants from both treatment groups were similar in terms of PD medications, PD features, H&Y scale, and psychosocial and cognitive measures at baseline.

There were no adverse events attributed to the vitamin D supplementation. Three participants (10%) were lost to follow-up due to loss of contact (n=2) and illness in the family (n=1). Two of these losses were from the placebo group and one was from the treatment group. One individual from each group was lost before 3 months, and the remaining loss occurred from the placebo group, before the 6 month visit.

Effect of Vitamin D Supplementation Group on Motor Symptoms

There were no consistent patterns or trends for any of the outcomes in the plots showing motor outcomes of interest (Timed 8 Meter Walk, TUG during both the “on” and “off” states, and UPDRS III subscore during both the “on” and “off” states) over time

for each individual (data not shown). When mean scores were plotted over time, most outcome variables showed similar trends for both treatment groups (Figures 3-5). However, TUG during the "off" state did show diverging trends for the two groups. The placebo group took an average of 2.7 seconds longer to complete TUG over time, whereas the treatment group showed a mean improvement of ~1.4 seconds from baseline to 6 months (Figure 4). Table 3 shows the mean values at each time point and the percent change from baseline to 6 months. Timed 8 Meter Walk and TUG during the "on" state, had very small percent changes (< 5%). The percent change in mean UPDRS III score during the "off" state was somewhat larger (10-11%), but it was of similar magnitude for both treatment groups. UPDRS III during the "on" state had a larger percent change than the other outcomes (27-31%), but the change was in the same direction and of similar magnitude for both groups. Furthermore, whereas the change was in the positive direction for UPDRS III during the "off" state, it was in the negative direction during the "on" state. As illustrated in Figure 4, TUG in the "off" state was the most different between the two groups, with a 12% decrease in time for the treatment group to complete TUG and a 24% increase in time to complete the task for the placebo group (Table 3). Results from the repeated measures ANOVA analysis (Table 3 in the Appendix) show no statistically significant improvement for the treatment group over placebo group, for any of the motor outcomes over time. The p-values for the Timed 8 Meter Walk, TUG during "on" and "off" state, and UPDRS III during "off" state ranged from 0.18-0.75. Results for the repeated measures analysis of UPDRS III during the "on" state had a corresponding p-value bordering on statistically significant (p=0.08); however, when looking at the plot, it is apparent that whereas the treatment and placebo groups diverged

at the 3 month visit, the two groups ended up with very similar final scores at 6 months (Figure 5).

Effect of Vitamin D Supplementation on Serum 25(OH)D Concentration

The mean serum 25(OH)D concentrations over time and the mean change in 25(OH)D over visits for each group are shown in Tables 4 and 5, respectively. There was almost no change in mean 25(OH)D concentrations over time for the placebo group (range 24.9-25.9 ng/ml). The treatment group showed a very large, statistically significant increase in mean 25(OH)D concentration of ~48 ng/ml within three months ($p=0.0005$). After 3 months, the treatment group's mean serum concentration leveled off. It was also determined that the two groups (treatment and placebo) did not have a significant difference in serum vitamin D concentrations at baseline ($p=0.15$). There was a statistically significant difference between the two groups' concentrations at 3 months and 6 months, however ($p=0.001$ and $p=0.0005$, respectively).

Heaney et al. reported that, in healthy individuals, 25(OH)D concentrations rise about 0.7 nmol/L for every 40 IU vitamin D (28), which equates to an increase of ~0.28 ng/ml for every 40 IU. In the ViDiP Pilot Study, the treatment group showed an increase of 0.25 ng/ml per 40 IU and the placebo group showed an increase of 0.13 ng/ml per 40 IU, over the first 3 months (Table 6). Table 7 shows the number of study participants who reached concentrations of serum 25(OH)D that are considered adequate. Whereas only 50% of both groups had serum concentrations greater than or equal to 20 ng/ml at baseline, 100% of the treatment group and 83% of the placebo group had reached this concentration after 3 months. A relatively small percentage of both groups (19-29%)

began with concentrations of greater than or equal to 30 ng/ml. Within 3 months, 100% of the treatment group had reached this concentration, but the proportion of the placebo group meeting this guideline did not change.

Dose-response Association between Serum 25(OH)D Concentration and Motor Symptoms

Results of the linear regression analysis of serum 25(OH)D concentration and motor symptoms are shown in Table 8. Adjusting for the potential confounding factors (age, BMI, gender, and length of time since PD diagnosis) did not meaningfully change the parameter estimates. Both the unadjusted and multivariate models yielded statistically non-significant regression coefficients of less than zero for most motor outcomes of interest (Timed 8 Meter Walk, TUG “on”, and UPDRS III “on” and “off”). However, the unadjusted and adjusted model for TUG “off” yielded a borderline significant (p=0.05) estimate. This model estimates that for every one ng/ml increase in 25(OH)D, the average time to complete TUG “off” will decrease by approximately 0.04 seconds. Looking at the performance results for the different motor tasks in Table 8, TUG “off” is the only motor outcome that appeared to improve, with decreasing time required to complete the task, as serum concentration category increased. The other motor outcomes had no consistent patterns or trends.

DISCUSSION

Summary

The primary aim of this study was to assess whether high dose vitamin D supplementation would improve PD-related motor symptoms. To investigate the treatment effect of high dose oral vitamin D supplementation on motor symptoms, three motor outcomes of primary interest were measured at baseline, 3 months, and 6 months. In this preliminary analysis, the treatment did not significantly improve performance on these motor tasks over time, relative to the placebo group. One motor outcome, TUG during the "off" state, did show a mean improvement of ~1.4 seconds from baseline to 6 months for the treatment group, whereas the placebo group took an average of 2.7 seconds longer to complete the test. However, this difference between the groups, over time, was not statistically significant ($p=0.54$) and could be the result of chance variability.

Another topic explored in this study was the effect of the vitamin D supplementation on serum 25(OH)D concentration. The placebo group, who was taking 600 IU/ day oral vitamin D supplement, had no statistically or meaningfully significant change in 25(OH)D concentration over the 6 month study period. The treatment group, who was receiving ~7743 IU/day, had a very large and statistically significant increase in serum 25(OH)D within 3 months, followed by a plateau effect within 6 months. Additionally, the treatment group had an overall increase in serum 25(OH)D concentration similar to what has been reported for healthy populations. On the other hand, the placebo group's increase per unit of vitamin D supplement was less than half

that of healthy populations (0.07-0.13 ng/ml increase per 40 IU vitamin D, compared to 0.28 ng/ml increase per 40 IU in healthy populations). This suggests that patients with PD respond to vitamin D supplement similarly to the general population at very high doses, but not at doses slightly higher than those found in multivitamin supplements. IOM defines vitamin D adequacy as 20 ng/ml (18). Half of both groups began the study with concentrations of 20 ng/ml or greater; everyone (100%) in the treatment group reached and sustained this "adequate" level, whereas the placebo group was more variable (83% at 3 months and 75% at 6 months) over the course of the study. As several sources cite 30 ng/ml as necessary for bone health, this commonly used cut point for sufficiency was also examined. Nineteen percent of the treatment group started with a concentration of 30 ng/ml or greater and 100% reached this level within 3 months. In contrast, the percentage of the placebo group with concentrations of 30 ng/ml or greater remained approximately the same throughout the study (~25-30%).

This study also considered the effect of actual serum 25(OH)D concentration, rather than treatment group status, on PD-related symptoms. The association for only one motor outcome (TUG during the "off" state) was borderline statistically significant. For this outcome, a one ng/ml increase in serum 25(OH)D was associated with an average 0.04 second improvement for the test. Consistent with this result, the pattern of results indicated a monotonic decrease in time to complete the TUG task with each increasing quartile of serum 25(OH)D. The results from the remaining regression models were consistent with no association between serum 25(OH)D and motor outcomes. Despite this lack of association, it can be speculated that the beneficial effects of vitamin D may only be noticeable in PD patients during the "off" medication state, when the drugs are not

working and there is more room for improvement.

Strengths and Limitations

The greatest strength of this study was its design. To our knowledge, this is the only randomized, double-blind, active-placebo-controlled trial to have assessed the effect of vitamin D supplementation on PD-related motor symptoms in patients with PD. This design helped reduce the likelihood of bias and enabled the consideration of multiple outcomes. Another strength of this study was that the majority of the outcomes of primary interest, including the Timed 8 Meter Walk, TUG "on," TUG "off," and serum 25(OH)D concentration, were all objective measures, which further limited biases. TUG has been found to have excellent interrater [intraclass correlation coefficient (ICC)=0.99] and intrarater reliability (ICC=0.99) and good test-retest reliability in persons with PD (27).

The results, however, should be interpreted with caution. Because this was a preliminary analysis of pilot data with a small sample size (n=30), the study had limited statistical power. Further, because this was a pilot study, formal sample size power estimates were completed only for the first primary outcome measure—mean and median serum 25(OH)D concentrations. Additional limitations involved one of the outcome variables of primary interest—UPDRS part III. This was a subjective measure; instead of a direct measurement, the ratings were an assessment based on perception of symptoms, which may have introduced bias. Also, there were many missing values for this variable, which limited the power of the model. It should also be noted that while compliance rates were extremely high for the weekly pill, the compliance rates for the daily supplement

were less consistent.

Future Directions

This pilot clinical trial is still underway and once it is complete, a final analysis will be performed. The resulting larger sample size will provide greater statistical power and allow for better detection of any statistically significant differences between the treatment groups. It will consider additional motor outcomes to further assess the potential effect of supplementation dose on PD-related motor symptoms and also assess the relationship between vitamin D supplementation and non-motor symptoms of PD. Considering the lack of association between serum 25(OH)D concentration and motor symptoms in this study, future investigations may want to focus on possible neuroprotective effect of vitamin D in PD, rather than symptomatic effect.

Because the IOM recommended vitamin D supplementation dose of 600 IU/day did not successfully raise all placebo group participants' serum 25(OH)D concentrations to the "adequate" level of 20 ng/ml, these guidelines may not be appropriate for persons with PD. Further studies may indicate whether guidelines tailored to specific chronic diseases and/or risk factors would be beneficial.

Based on the results from this study, a supplementation dose of 50,000 IU/week is more than sufficient for patients with PD to reach a serum 25(OH)D concentration of 30 ng/ml. Further studies are needed to determine if there is an intermediate dose, between the 50,000 IU/week and the 600 IU/day that is sufficient for patients with PD to reach the recommended 25(OH)D concentration.

Conclusion

While there is biological plausibility suggesting that vitamin D insufficiency could play a role in the worsening of motor symptoms of patients with PD, this study was not able to support such a theory. The results from this pilot clinical trial suggest that vitamin D supplementation has minimal impact on PD-related motor symptoms, as there was no significant improvement for the treatment group, compared to the placebo group, for any of the motor outcomes of interest. In considering the association between serum 25(OH)D concentration, regardless of treatment group, and motor symptoms, there also appears to be very limited impact. There was a marginally statistically significant association for only one motor outcome (Timed-Up-and-Go during the "off" state). Increasing quartiles of serum 25(OH)D were associated with quicker times to complete the TUG task. When interpreting these results, it is important to consider the possibility that any symptomatic benefits of vitamin D on motor outcomes during the "on" medication state are dwarfed by the symptomatic response of PD medications, coupled with inter-individual variability. Finally, the mean change in 25(OH)D in patients with PD taking 54,200 IU/week supplemental vitamin D was consistent with previous findings in healthy men, of 0.7 nmol/L for every 40 IU/day; most of this change occurred within 3 months. However, patients taking doses consistent with Institute of Medicine (IOM) recommendations (600 IU/day) did not experience a significant change in 25(OH)D concentrations. Current IOM recommendations for adequate intake may be insufficient for patients with PD.

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TABLES

Table 1. Sociodemographic Characteristics of Participants from the Pilot Study of Vitamin D Repletion in Patients with Parkinson's Disease

	Total Population[a] (n=30)	**Treatment Group (n=16)**	**Placebo Group (n=14)**
Age, year (SD[b], range)	64 (7.9, 44-75)	65 (7.3, 49-75)	64 (8.9, 44-72)
Men, n (%)	19 (63.3)	11 (68.8)	8 (57.1)
Race, n (%)			
White	26 (86.7)	13 (81.3)	13 (92.9)
Black	4 (13.3)	3 (18.7)	1 (7.1)
Education, n (%)			
High School Graduate	5 (16.7)	1 (6.3)	4 (28.6)
Some College	5 (16.7)	3(18.7)	2 (14.3)
Associate Degree	1 (3.3)	0 (0.0)	1 (7.1)
Bachelor Degree	10 (33.3)	6 (37.5)	4 (28.6)
Graduate Degree	9 (30.0)	6 (37.5)	3 (21.4)

[a] Sociodemographic characteristics not significantly different between the two groups (p-value > 0.05).
[b] SD=Standard Deviation

Table 2. Baseline Characteristics of Participants from the Pilot Study of Vitamin D Repletion in Patients with Parkinson's Disease

	Treatment Group (n=16)	Placebo Group (n=14)
BMI[a], mean (SD[b])	29.3 (3.3)	28.2 (5.6)
BMI < 30, n (%)	10 (60.0)	11 (78.6)
BMI ≥ 30, n (%)	6 (40.0)	3 (21.4)
25-hydroxyvitamin D concentration at baseline, ng/ml (SD, range)	20.2 (8.6, 6.7-35.2)	24.9 (8.6, 13.0-41.9)
Medications (Category), n (%)[c]		
Carbidopa/Levodopa	13 (81.3)	11 (78.6)
Dopamine agonists	8 (50.0)	11 (78.6)
Anticholinergics	2 (12.5)	1 (7.1)
MAO-B inhibitors	5 (31.3)	5 (35.7)
COMT inhibitors	5 (31.3)	1 (7.1)
Other	9 (56.3)	5 (35.7)
PD[d] Features, n (%)[e]		
Bradykinesia	8 (50.0)	10 (71.4)
Resting Tremor	11 (68.8)	9 (64.3)
Rigidity	8 (50.0)	8 (57.1)
Postural Instability	4 (25.0)	4 (28.6)
Hoehn and Yahr Scale, n (%)		
Stage 1.0	3 (18.7)	2 (14.3)
Stage 1.5	1 (6.3)	2 (14.3)
Stage 2.0	5 (33.3)	3 (21.4)
Stage 2.5	3 (18.7)	2 (14.3)
Stage 3.0	3 (18.7)	5 (35.7)
Stage 3.5	0 (0.0)	0 (0.0)
Stage 4.0	1 (6.3)	0 (0.0)
MMSE[f] Score, mean (SD, range)	28.6 (1.9, 25-30)	28.1 (1.9, 25-30)
GDS[g] Score, mean (SD, range)	3.3 (3.0, 0-11)	3.6 (3.3, 0-13)
Beck Anxiety Score, mean (SD, range)	7.8 (3.9, 0-16)	11.4 (7.5, 1-30)
BDS[h], mean (SD, range)	6.4 (5.0, 0-19)	12.9 (12.2, 2-46)

[a] BMI=Body Mass Index
[b] SD=Standard Deviation
[c] Many patients take multiple medications, categories not mutually exclusive.
[d] PD=Parkinson's Disease
[e] Categories not mutually exclusive.
[f] MMSE=Mini Mental State Examination
[g] GDS=Geriatric Depression Scale
[h] BDS= Beck Depression Score

Table 3. Results for Each Motor Outcome of Interest, By Treatment Group, at Baseline and Follow-up

Mean Time [seconds (SD[a])] to Complete Timed 8 Meter Walk				
	Baseline	3 Month	6 Month	Percent Change[b]
Treatment Group	17.2 (7.6)	17.9 (7.9)	17.8 (10.2)	3.5
Placebo Group	17.8 (6.8)	19.0 (7.0)	18.4 (6.6)	3.4
Mean Time [seconds (SD)] to Complete TUG[c] During "On" Medication State[d]				
	Baseline	3 Month	6 Month	Percent Change
Treatment Group	10.0 (2.4)	9.9 (1.9)	9.8 (2.2)	-2.0
Placebo Group	11.2 (5.1)	11.7 (5.6)	11.4 (3.1)	1.8
Mean Time [seconds (SD)] to Complete TUG During "Off" Medication State[e]				
	Baseline	3 Month	6 Month	Percent Change
Treatment Group	11.6 (4.8)	10.5 (1.9)	10.2 (2.0)	-12.1
Placebo Group	11.5 (4.6)	13.6 (7.6)	14.2 (8.0)	23.5
Mean UPDRS[f] Part III Subscore (SD) During "On" Medication State				
	Baseline	3 Month	6 Month	Percent Change
Treatment Group	15.6 (10.1)	18.5 (14.6)	10.7 (9.5)	-31.4
Placebo Group	16.5 (12.0)	12.2 (9.6)	12.1 (11.1)	-26.7
Mean UPDRS Part III Subscore (SD) During "Off" Medication State				
	Baseline	3 Month	6 Month	Percent Change
Treatment Group	21.0 (10.8)	24.3 (15.6)	23.3(13.5)	11.0
Placebo Group	21.8 (10.4)	22.4 (11.4)	24.0 (10.6)	10.1

[a] SD=Standard Deviation
[b] Percent change is from baseline to 6 month visit
[c] TUG=Timed-Up-and-Go
[d] "On" Medication State refers to when the patient is having a good response to medication and minimal symptoms.
[e] "Off" Medication State describes when medication is not working.
[f] UPDRS=Unified Parkinson's Disease Rating Score

Table 4. Mean 25-Hydroxyvitamin D Concentrations over Time

Group	Baseline[a]	3 Months[b]	6 Months[c]
Treatment Group, ng/ml (SD[d])	20.2 (8.6)	69.1 (41.7)	71.9 (38.3)
Placebo Group, ng/ml (SD)	24.9 (8.6)	25.9 (9.1)	25.5 (7.5)

[a] Treatment group (n=16), Placebo group (n=14)
[b] Treatment group (n=15), Placebo group (n=12)
[c] Treatment group (n=14), Placebo group (n=12)
[d] SD=Standard Deviation
[*] Missing samples for 1 placebo subject at 3 months and 1 treatment subject at 6 months

Table 5. Mean Changes in 25-Hydroxyvitamin D Concentrations over Time

	Change in ng/ml, mean (SD[a])	p-value
Treatment Group		
Baseline to 3 Months (n=15)	48.1 (41.3)	0.0005
Baseline to 6 Months (n=14)	50.2 (39.4)	0.0004
3 Months to 6 Months (n=14)	2.2 (36.3)	0.8221
Placebo Group		
Baseline to 3 Months (n=12)	2.0 (5.9)	0.2579
Baseline to 6 Months (n=12)	1.0 (7.3)	0.6433
3 Months to 6 Months (n=11)	-2.2 (4.8)	0.1685

[a] SD=Standard Deviation

Table 6. Mean Change in 25-Hydroxyvitamin D Concentrations (ng/ml) per 40 IU Oral Vitamin D Supplement

	Within 3 Months	Within 6 Months
Treatment Group	0.25	0.26
Placebo Group	0.13	0.07

*Heaney et al. found an average change of 0.28 ng/ml per 40 IU Vitamin D daily (28).

Table 7. Number of Study Participants Who Reached "Adequate[a]" Levels of Serum 25-Hydroxyvitamin D

	Baseline[b]	3 Month[c]	6 Month[d]
Serum Concentration ≥ 20 ng/ml			
Treatment Group			
n (%)	8 (50.0)	15 (100.0)	14 (100.0)
Placebo Group			
n (%)	7 (50.0)	10 (83.3)	9 (75.0)
Serum Concentration ≥ 30 ng/ml			
Treatment Group			
n (%)	3 (18.8)	15 (100.0)	13 (92.9)
Placebo Group			
n (%)	4 (28.6)	3 (25.0)	4 (33.3)

[a] Institute of Medicine defines adequate levels of vitamin D as 20 ng/ml; other research suggests 30 ng/ml is required for bone health.
[b] Treatment group (n=16), Placebo group (n=14)
[c] Treatment group (n=15), Placebo group (n=12)
[d] Treatment group (n=14), Placebo group (n=12)
[*] Missing samples for 1 placebo subject at 3 months and 1 treatment subject at 6 months

Table 8. Serum 25-Hydroxyvitamin D Concentrations and Motor Outcomes

Mean Results from Motor Outcomes[a]					
Range of Serum 25(OH)D Concentrations (ng/ml) per Quartile	**Timed 8 Meter Walk (seconds)**	**TUG**[b] **During "On" Medication State**[c] **(seconds)**	**TUG During "Off" Medication State**[d] **(seconds)**	**UPDRS**[e] **During "On" Medication State (score)**	**UPDRS During "Off" Medication State (score)**
Q1: 0.0-20.0	19.8	11.2	13.6	12.9	23.5
Q2: 20.1-30.0	18.2	11.8	13.2	13.2	19.8
Q3: 30.1-45.0	15.4	9.5	10.4	14.9	23.3
Q4: 45.1+	18.5	9.7	9.9	14.5	22.9
Parameter Estimate[f] **(p-value)**					
Unadjusted Model	-0.030 (0.25)	-0.014 (0.30)	-0.036 (0.05)	-0.028 (0.52)	-0.050 (0.23)
Multivariate Model[g]	-0.024 (0.29)	-0.011 (0.37)	-0.033 (0.05)	-0.038 (0.38)	-0.053 (0.17)

[a] Least squares means calculated from unadjusted linear regression model.
[b] TUG=Timed-Up-and-Go test
[c] "On" Medication State refers to when the patient is having a good response to medication and minimal symptoms.
[d] "Off" Medication State describes when medication is not working.
[e] UPDRS=Unified Parkinson's Disease Rating Score
[f] Beta coefficient from linear regression that modeled serum 25(OH)D as a continuous variable
[g] Multivariate model adjusted for the following factors: age, body mass index, gender, and length of time since Parkinson's disease diagnosis

FIGURES

Figure 1. Study Schema

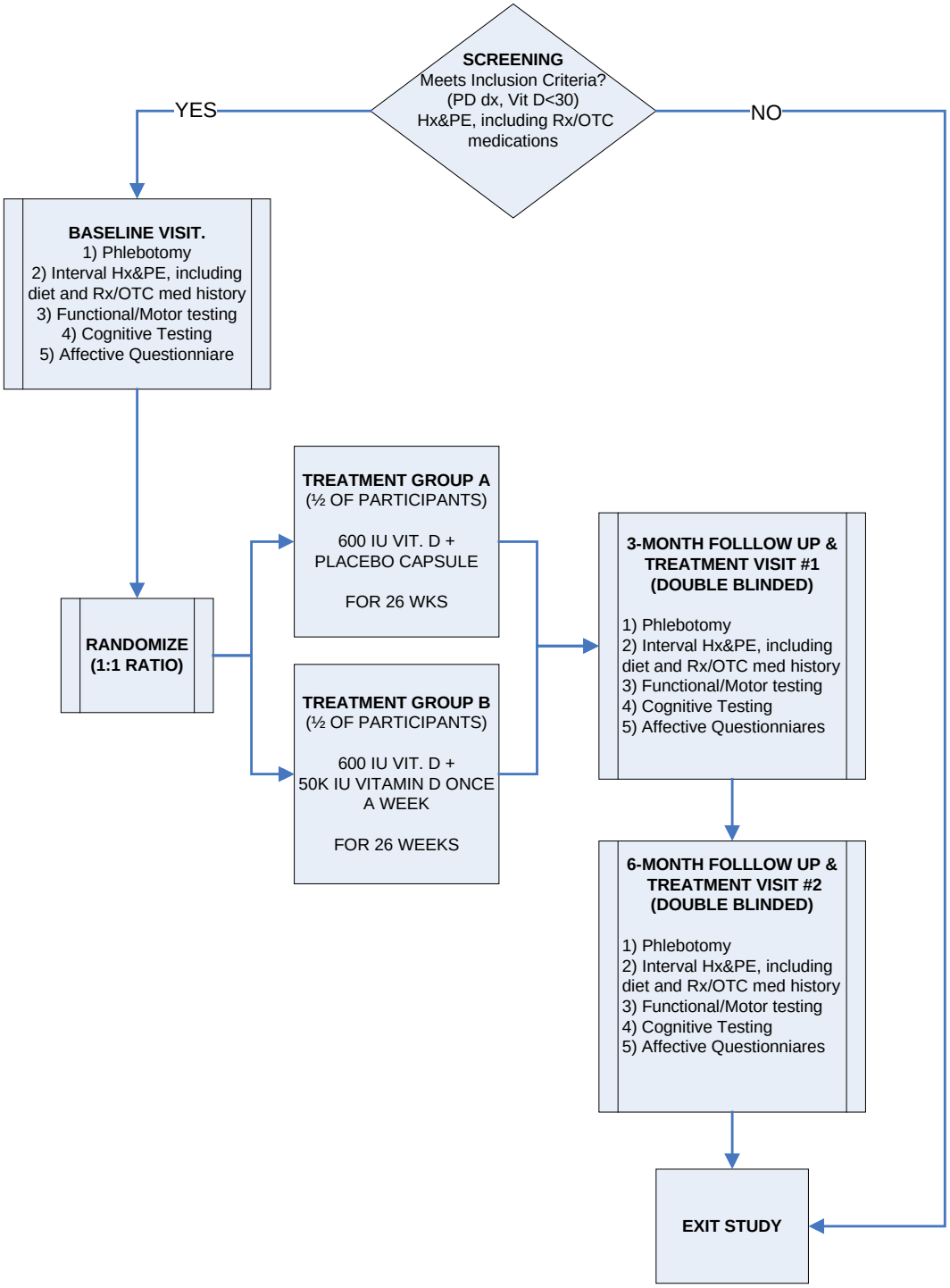


Figure 2. Study Flow Diagram

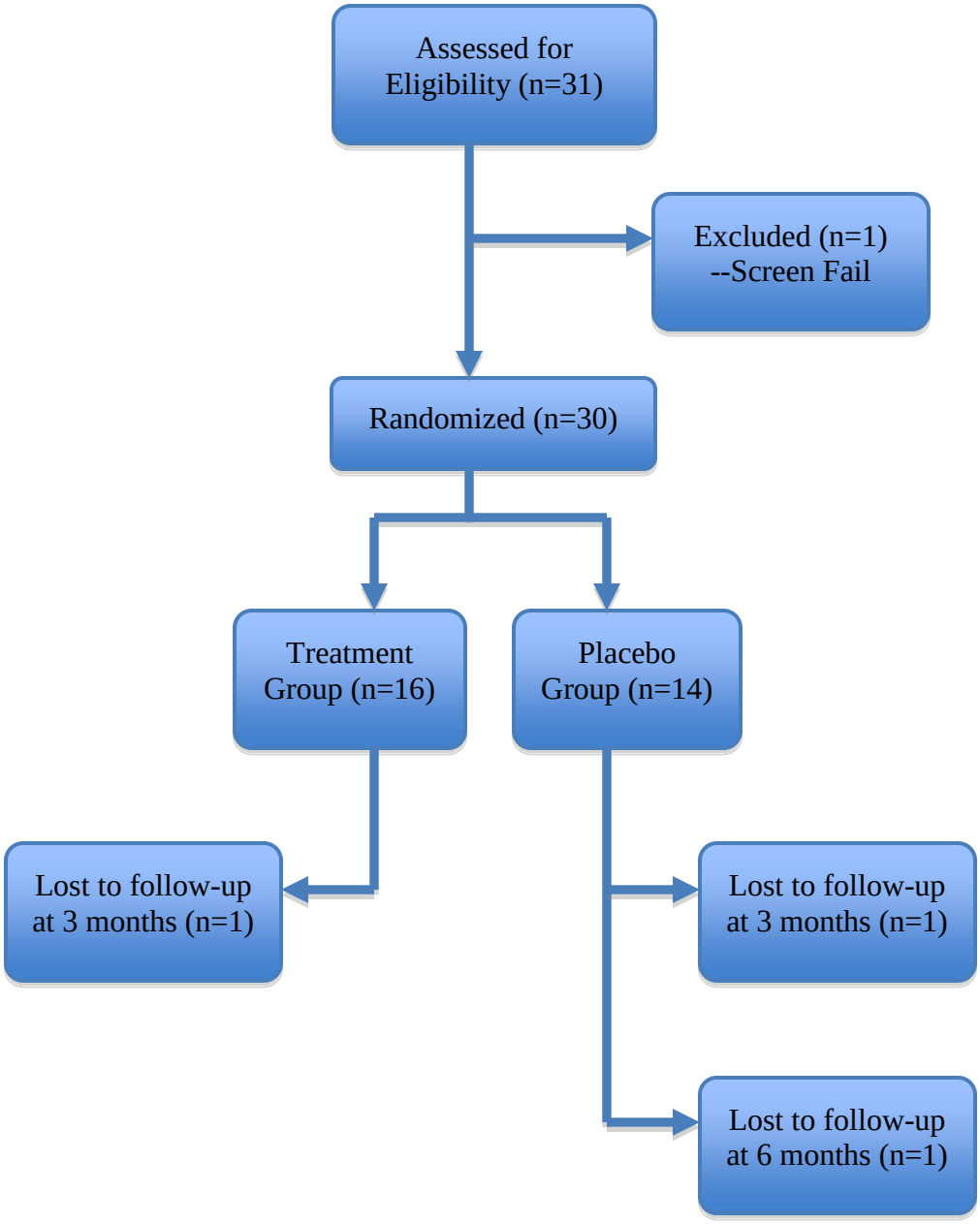
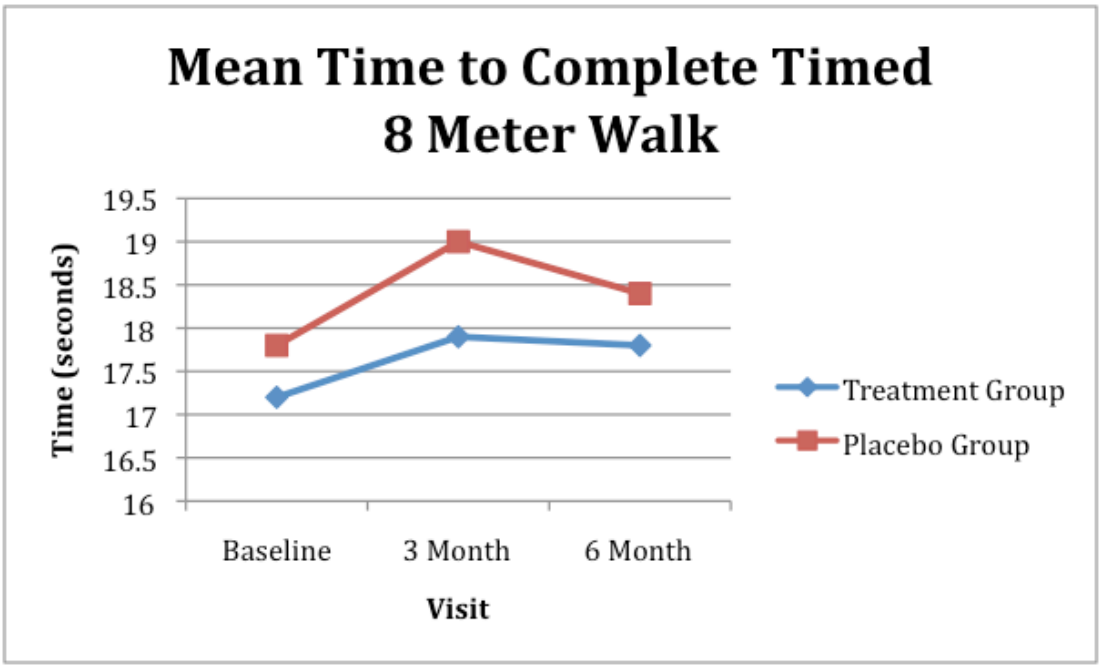


Figure 3


Mean Time to Complete Timed 8 Meter Walk
Time (seconds)
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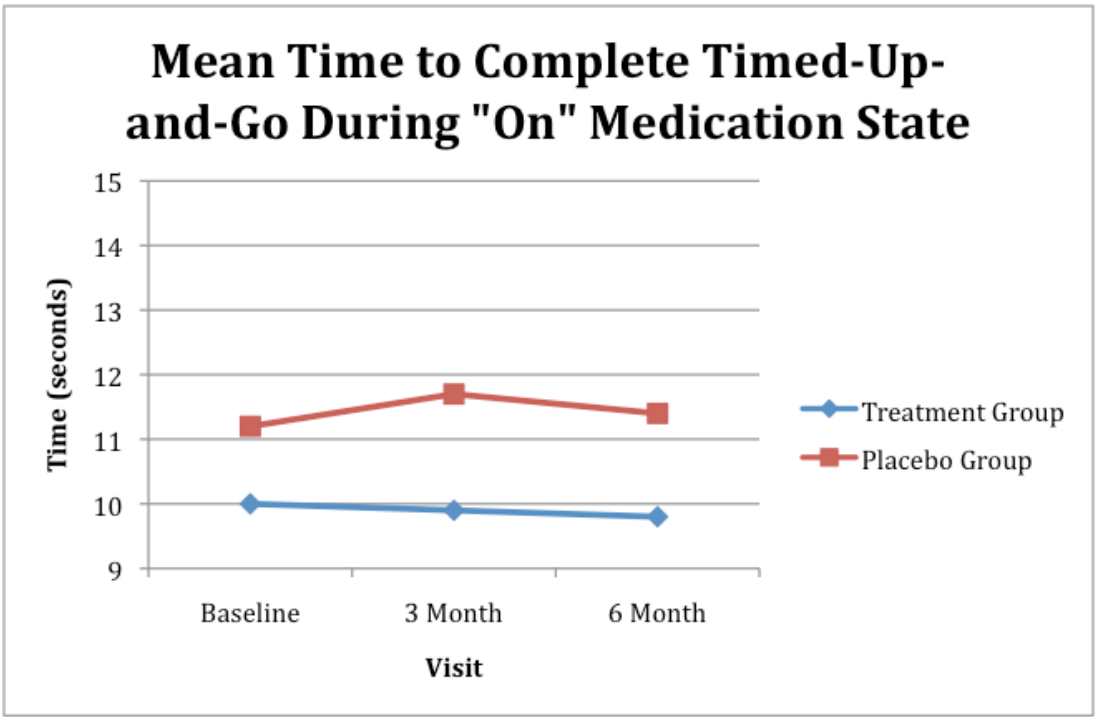
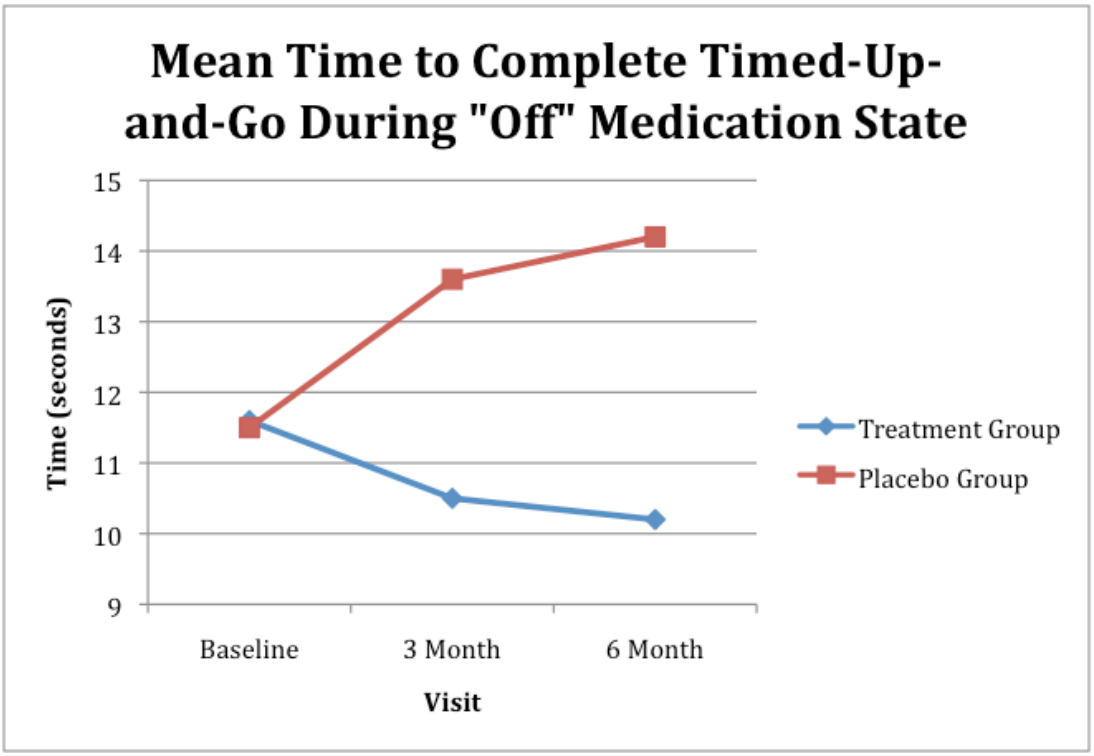
19
18.5
18
17.5
17
16.5
16
Baseline
3 Month
6 Month
Visit
Treatment Group
Placebo Group

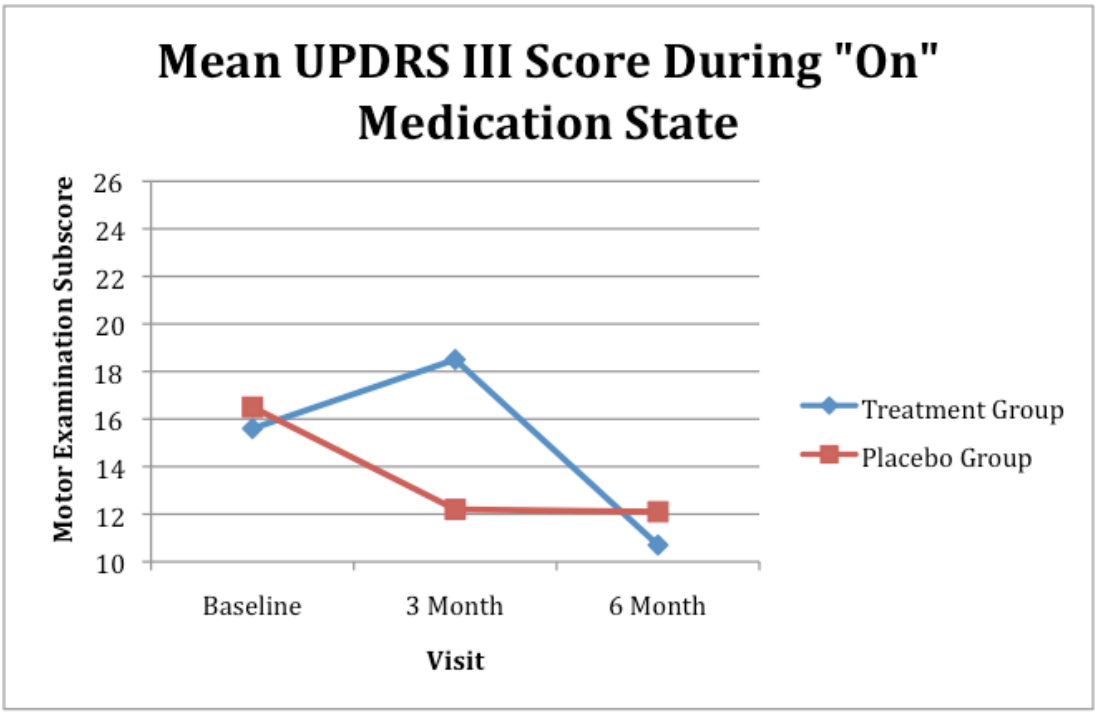
Figure 4

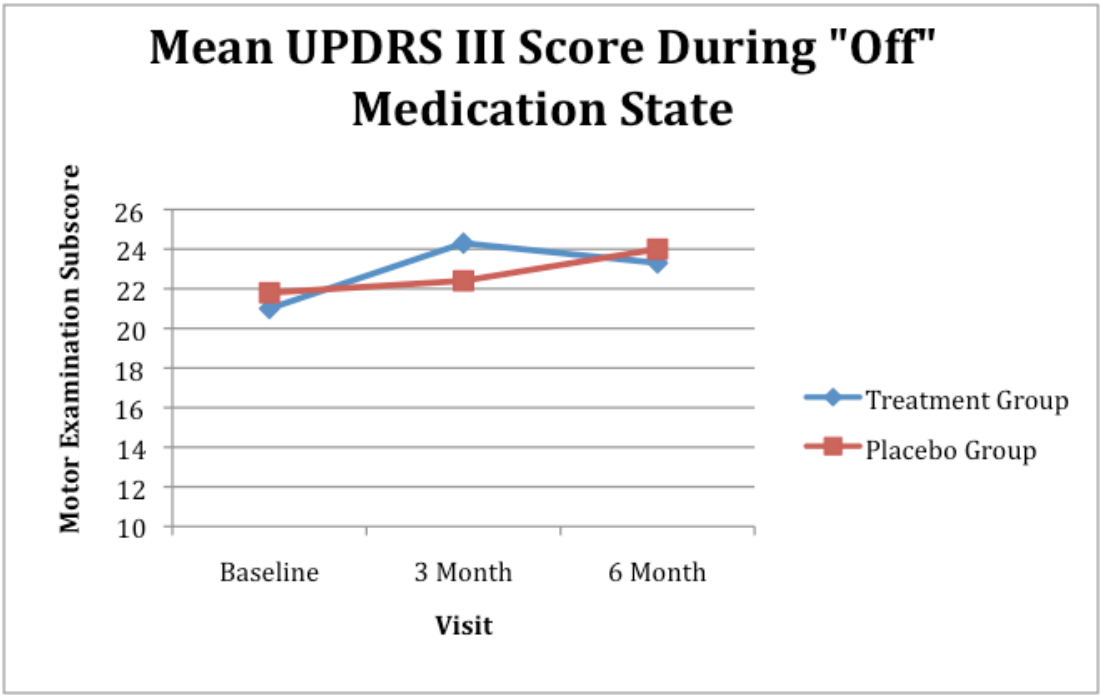




* "On" Medication State refers to when the patient is having a good response to medication and minimal symptoms and "Off" Medication State describes when medication is not working.

Figure 5





*UPDRS=Unified Parkinson's Disease Rating Scale
** "On" Medication State refers to when the patient is having a good response to medication and minimal symptoms and "Off" Medication State describes when medication is not working.

APPENDIX

The determination of statistical power for this study's analyses was based on an independent group's t-test comparing the mean change from baseline to a follow-up visit between the two treatment groups. For these analyses, it was assumed that the mean vitamin D concentration at baseline for PD patients is similar to that found in a chart review (22.5 ng/ml), and that the high-dose vitamin D supplement will increase this value to > 42 ng/m, but that the low-dose supplementation will not have this effect (25).

For the timed walking task, the specification of the effect size for vitamin D supplementation was based on data from NHANES III (14) which indicated that for normal individuals, the time to walk 8 feet correlated significantly with vitamin D concentration; individuals whose vitamin D concentrations were 13.5 ng/ml take 0.6 seconds longer to walk 8 feet than individuals whose vitamin D concentrations were > 42 ng/ml. The estimate for the standard deviation of the change from baseline to follow-up for the time to walk 8 meters came from data by Brusse (30) which showed that the mean ± standard deviation (SD) for comfortable gait speed was 0.91 ± 0.21 m/sec. Extrapolating to 8 meters, the mean ± SD is 7.3 ± 1.7 seconds. Assuming that the standard deviation would be the same at baseline and follow-up (1.7 sec) and that the correlation between the walking times at the two time points would be 0.5, the standard deviation for the change in walking time from baseline to follow-up would also be 1.7 sec. For the independent groups t-test with 50 PD patients per group, with probability of a Type I error = 0.05 (two sided), and with the standard deviation of 1.7 in both groups, the power to detect a 0.6 second difference in the mean time to walk 8 meters between the

high-dose and low-dose supplementation groups is 0.41. The study has power = 0.8 to detect a 1.0 second difference between the means of the two groups (25).

Table 1. Correlations of variables with Serum 25-Hydroxyvitamin D concentrations

	Serum 25(OH)D, r (p-value)
BMI[a]	-0.08 (0.69)
Ln[b](Age)	-0.23 (0.21)
Race	*0.48 (0.01)
Gender	0.15 (0.44)
Season	-0.00 (0.99)
Length Since PD Symptoms	-0.27 (0.23)
Length Since PD Diagnosis	-0.07 (0.73)

[a] BMI=Body Mass Index
[b] Ln=Natural log
*The significant correlation between race and serum 25-hydroxyvitamin D concentrations in this population is expected, as the literature consistently reports lower concentrations in black versus white individuals.

Table 2. Average Serum 25-Hydroxyvitamin D Concentrations at Baseline by Race

Race	Serum 25(OH)D, ng/ml (SD[a])
White	24.0 (8.1)
Black	11.8 (4.8)

[a] SD=Standard deviation

Table 3. Results from Repeated Measures Linear Mixed Models for Each Motor Outcome of Interest

	F-Statistic	P-Value
Timed 8 Meter Walk		
Tx	0.05	0.83
Visit	2.69	0.09
Tx*Visit	1.80	0.18
Timed-Up-and-Go During "On" Medication State[a]		
Tx	0.83	0.37
Visit	1.78	0.19
Tx*Visit	0.36	0.70
Timed-Up-and-Go During "Off" Medication State[b]		
Tx	0.04	0.84
Visit	1.21	0.32
Tx*Visit	0.29	0.75
UPDRS[c] Part III Subscore During "On" Medication State		
Tx	0.08	0.78
Visit	1.67	0.21
Tx*Visit	2.80	0.08
UPDRS Part III Subscore During "On" Medication State		
Tx	0.01	0.94
Visit	0.79	0.46
Tx*Visit	0.32	0.73

[a] "On" Medication State refers to when the patient is having a good response to medication and minimal symptoms.

[b] "Off" Medication State describes when medication is not working.

[c] UPDRS=Unified Parkinson's Disease Rating Score