Bioavailable Vitamin D and Fall Risk:
The Osteoporotic Fractures in Men Study

A Thesis

by

Jennifer Cai Gillis

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CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Jennifer Cai Gillis

has been approved

Mentor/Advisor

Member

Member

Member

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Abstract

Fall Risk in Relation to Bioavailable Vitamin D: The Osteoporotic Fractures in Men (MrOS) Study

Authors: J Cai Gillis, Lynn Marshall, Jodi Lapidus, Iva Miljkovic, Amy H Warriner, Jeffrey R Curtis, Tien Dam, James M Shikany, Peggy M Cawthon, Rene Chun, Martin Hewison, Michael Nevitt, Jane A Cauley, Eric Orwoll, Carrie M Nielson

Purpose: Bioavailable 25-hydroxyvitamin D (bio25(OH)D) is that fraction of total 25(OH)D not bound to vitamin D binding protein (DBP). However, little is known about effects of bio25(OH)D on health outcomes. Vitamin D's potential effects on neurological and muscular outcomes might manifest in a higher fall risk among those with lower levels of bio25(OH)D. In this study, we evaluated associations of bio25(OH)D and total 25(OH)D with risk of falls in elderly men.

Methods and Results: The MrOS study enrolled 5,994 ambulatory men ≥65 years old from March 2000 to April 2002 at 6 sites in the US. Total 25(OH)D assays were performed in serum obtained at enrollment from 1,608 randomly chosen participants. Of these, a random sample of 678 was selected for additional assays to estimate free and bio25(OH)D.

Bio25(OH)D levels were estimated using validated mathematical models that account for DBP concentration and genotypically determined binding affinity. Falls were self-reported every 4 months by mailed questionnaires during 12 months of follow-up. We used log-binomial regression models to obtain relative risk (RR) estimates for any fall in relation to bio25(OH)D quartile. All models were adjusted for age, BMI, race, season of blood draw and latitude of clinic site. Potential confounders included medications, self-reported comorbidities and physical activity; confounding was assessed by a change in the RR of ≥10%. Four participants were missing fall information.

In the sample of 674, the mean (±sd) for bio25(OH)D was 12.98 (±5.65) nmol/L and for total 25(OH)D was 62.2 (±19.9) nmol/L. A fall was reported by 195 men (29%). Bio25(OH)D was correlated with total 25(OH)D (r=0.55) and fall risk did not significantly vary according to total 25(OH)D quartile.

Regression analysis showed a 0.65 (RR) for the 3rd quartile of bio25(OH)D compared to the highest (95% CI: 0.45-0.96). No significant association was observed for the two lowest quartiles of bio25(OH)D compared to the highest quartile. No additional confounders were identified among those examined. Further examination using spline analyses revealed a cubic relationship between bioavailable 25(OH)D and falls that offered no clear pattern for a range of bioavailable 25(OH)D levels associated with a lowered risk of falls

Conclusions: Bio25(OH)D was more strongly associated with any falls than total serum 25(OH)D when divided into quartiles. Spline analyses revealed a more biologically plausible nonlinear U-shaped curve for Total 25(OH)D than the cubic curve observed for bio25(OH)D. However, the spline for bio25(OH)D was suggestive of a possible linear relationship other than the dip in the middle therefore future research should further examine linearity in the association between bio25(OH)D and falls.

Introduction

The number of adults hospitalized for a fall related injury increased by 50% from 2001 to 2008.[1] In 2010, more than 2.3 million patients over 65 were treated in an emergency department for an injury sustained during a fall [2]. Falls in the elderly often result in head injuries, disability, the need for long term care, or death. A third of older adults require assistance with their activities of daily living as a result of injuries obtained from a fall.[3] The expected rise in the proportion of adults over the age of 65 years in the coming years coupled with the high cost of fall related health care indicates that falls potentially represent a serious threat to already limited health care resources. In 2000, fall related health care costs in older populations totaled $19 billion and by 2020 these costs are projected to increase to $54.9 billion.[2, 4] In order to avert this rising financial burden and help older adults maintain their independence for as long as possible, it is important to uncover new factors associated with a lower risk of falling that are amenable to prevention.

Vitamin D has been implicated as a potentially important factor in various aging health outcomes including osteoporosis, cancer, fracture risk, cognitive dysfunction, cardiovascular disease and all cause mortality. [5-10] Given these reported associations, it has been hypothesized that the potential neurological and muscular effects of vitamin D could possibly affect fall risk since reduced muscle strength or physical function might increase the likelihood of falling. Aging populations are particularly vulnerable to vitamin D deficiency because of reduced sun exposure, a diminished ability of the skin to synthesize vitamin D, and a reduction in skeletal muscle vitamin D receptors.[11] An estimated one billion people worldwide are vitamin D deficient

with older individuals representing most of these cases.[12]

Many studies have examined the role that vitamin D potentially plays in reduction of fall risk, but have provided mixed results.[13-16] A study by Menant et. al. showed that vitamin D insufficiency was associated with poorer physical function in both men and women and with fall rate in men.[15] However a study by Sai et al failed to identify serum 25(OH)D levels as a significant predictor of incident or recurrent falls.[13] A recent Cochrane review examining interventions for preventing falls in older community dwelling adults concluded that overall vitamin D failed to reduce the risk of falling but might do so in those with lower baseline vitamin D levels.[17] While the United States Preventive Task Force has recommended vitamin D supplementation as a means of reducing fall risk, meta-analysis conclusions of the effectiveness of supplementation have remained inconclusive.[16, 18-21] A randomized controlled trial which effectively increased total circulating vitamin D by providing a one-time high-dose vitamin D supplement (500,000 IU of cholecalcifoerol) reported a higher increased rate of falls compared to placebo.[22]

While evidence of the association of vitamin D with falls has been inconclusive, thus far these studies have focused only on total vitamin D measures. Emerging evidence is suggestive that free or bioavailable vitamin D may potentially represent better estimates of vitamin D for certain health outcomes such as bone mineral density and mineral metabolism in advanced kidney disease, however to our knowledge no research currently exists examining these estimates in relation to falls.[23, 24] Free 25(OH)D is that portion of circulating 25(OH)D which is not bound to DBP or albumin and only represents approximately 1% of circulating vitamin D. [23]

Bioavailable 25(OH)D is the portion of free 25(OH)D in circulation as well as that which is albumin-bound and represents approximately 10-15% of the total circulating 25(OH)D.[23, 25] According to the free hormone hypothesis, only vitamin D metabolites which are unbound to vitamin D binding protein (DBP) are available to cells for biologic activities.[26] Estimates of the hepatic metabolite of vitamin D, 25-hydroxyvitamin D (25(OH)D), using total 25(OH)D do not take into account the binding affinity or amount of DBP and therefore may provide a less specific measure of biologically available circulating vitamin D than free or bioavailable 25(OH)D.

It is estimated that anywhere from 23% to 77% of 25(OH)D variation may be attributable to genetic factors.[27-29] By genotyping two polymorphisms in the DBP gene (rs7041 and rs4588), the binding affinity of DBP to 25(OH)D can be estimated. Vitamin D is mainly carried in the serum by DBP or albumin, with albumin acting as a lower affinity binder. When the binding affinity of DBP is high, the levels of free or bioavailable 25(OH)D are lower than with lower binding affinity DBP even if total 25(OH)D levels are equivalent.[26] This process works primarily by codon changes in the DBP genes mentioned previously which alter the isoelectric point of DBP and its affinity for 25(OH)D.[30] In rs4588, lower affinity for 25(OH)D results from a base pair change of ACG to AAG.[31] This base change substitutes the amino acid Lysine (Lys) for Threonine (Thr) on codon 436 (previously 420) resulting in a protein change from GC-1 to GC-2.[30, 31] Likewise when the base pair GAT changes to GAG at rs7041 the amino acid Aspartate (Asp) becomes Glutamate (Glu) at codon 432 (previously 416) causing a change to GC-1s which has a higher affinity than GC2 but a lower affinity than GC-1f.

The combination of the rs4588 and rs7041 genotypes results in six common haplotypes of DBP. Bioavailable 25(OH)D and Free 25(OH)D levels are estimated using validated mathematical models that take into account DBP concentration, albumin concentration, and haplotypic differences in DBP binding affinity. Since the binding of 25(OH)D to albumin is appreciably weaker than to DBP, albumin-bound 25(OH)D has the potential to be taken up by cells.[23] The implications for estimating measures of free and bioavailable 25(OH)D rather than total 25(OH)D are only beginning to be explored, but findings of two recent studies highlight the possible significance of a shift in focus to these measures. In a 2011 study by Powe et. al., free and bioavailable 25(OH)D levels were associated with bone mineral density in a cross-sectional analysis of healthy adults but total 25(OH)D levels were not.[24] Similarly in a 2012 study, bioavailable 25(OH)D but not total 25(OH)D was significantly associated with measures of mineral metabolism hemodialysis patients.[23] These findings indicate that free and bioavailable measures of 25(OH)D may have more clinical significance as a marker for health outcomes than total measures of 25(OH)D.

Since no research exists on the associations between free and bioavailable vitamin D with falls in elderly men, it is of interest to determine if these measures provide a stronger association than total vitamin D levels. The existing literature and preliminary analysis of the data used in this study suggests that bioavailable and free 25(OH)D are highly correlated (r=0.99), therefore this analysis focused exclusively on bioavailable 25(OH)D. Bioavailable 25(OH)D was selected over free 25(OH)D since albumin bound 25(OH)D has the potential to be taken up by cells given its weaker binding affinity. This prospective study examined associations between

baseline measures of bioavailable 25(OH)D with falls occurring within the first twelve months of study entry.

Additionally, it examined appendicular lean mass and physical function as possible mediators in the relationship between bioavailable 25(OH)D and falls. These variables were selected based on biologically plausible pathways of mediation between any observed association of vitamin D and falls. The finding that the nuclei of muscle cells contain vitamin D receptors indicates that vitamin D plays an important role in the growth and function of muscle tissue. [32] Further, knockout mice lacking vitamin D receptors have been shown to display altered muscular development giving further evidence to the importance of vitamin D in muscle growth and health.[33] Since expression of vitamin D receptors decreases with age, much of the decline in skeletal muscle and muscle strength seen in older populations is possibly related to a lowered ability to utilize vitamin D in cells. [32] Muscle strength and function is especially paramount in older populations since balance, strength and coordination are all important components to fall prevention.

Type II muscle fibers contract rapidly and therefore have been posited as important to fall prevention. These fibers have been shown to increase in biopsy following vitamin D supplementation, indicating that vitamin D likely plays an important role in maintaining the muscular and physical function that allows an individual to avoid falling. [34]

Several studies have examined the association of total vitamin D levels falls and physical function but have at times produced conflicting results. [21, 35-39] In a study of

4,100 ambulatory older adults aged 60 and older, vitamin D levels between 40 nmol/L and 94 nmol/L were associated with better physical function performance compared to levels below 40 nmol/L however little improvement was seen for levels exceeding 94 nmol/L. [35] Additionally, a 2007 study of 1155 adults 65 and older found that vitamin D levels were inversely associated with poorer physical performance. [39] However a systematic review by Latham et. al. in 2003 found no association between physical function and vitamin D. [21] Physical function has been associated with falls in elderly populations so should an association exist between bioavailable 25(OH)D and falls, it is of interest to examine if this association is mediated through physical function or lean mass.

Methods

Study Population

MrOS is a prospective cohort study of 5,994 ambulatory non-institutionalized men over 65 years of age. Details regarding enrollment for the MrOS study have been described elsewhere.[40, 41] Briefly, recruitment for the MrOS study was conducted from March 2000 through April 2002 at six locations across the United States. The six clinical sites were located in Portland, Oregon (Oregon Health and Science University); San Diego, California (University of California, San Diego); Birmingham, Alabama (University of Alabama at Birmingham); Minneapolis, Minnesota (University of Minnesota); Palo Alto, California (Stanford University); and Pittsburgh, Pennsylvania (University of Pittsburgh). The goals of MrOS are to study the risk of fracture in older men in relation to skeletal determinants, lifestyle and medical factors, and sex steroids.

Recruitment involved the identification of potential subjects in the clinic site areas using existing databases such as voter registrations, motor vehicle registrations and the Health Care Financing Administration database. Invitations to join the study were then mailed to these participants. Additionally, advertisements were placed in local newspapers as well as radio and television programs. Flyers were distributed to local senior organizations and presentations made at targeted senior gatherings.

5,908 participants of the original cohort had at least one vial of baseline serum and of these, 1,608 were randomly chosen for initial 25(OH)D assays. Of these 1,608 participants from the original vitamin D assays, a random sample of 678 were selected for new 25(OH)D assays which also measured vitamin D binding protein and represent the cohort used in this proposed analysis. Of these 678 cohort members, four were missing information related to falls within the first year of study entry therefore leaving an effective sample size of 674 for the present analysis.

Descriptive Characteristics

Self-reported information regarding age, race/ethnicity, alcohol and smoking history, fall history, medical history and medication history were recorded at baseline using a questionnaire. The self-reported questionnaire was then verified at the initial visit by a study team member who reviewed all answers with the participant to insure that responses were recorded accurately. Body mass index (BMI) and season of blood draw were also recorded at the baseline visit. Participants were classified as non-Hispanic white or other race/ethnicity for this analysis due to small numbers of racial/ethnic minority participants. Smoking status was dichotomized as “current or

former" versus "never". Clinic site location was divided into a binary variable based on high or low latitude. High latitudes included Portland (45°), Minneapolis (44°), and Pittsburgh (40°). Low latitudes included Palo Alto (37°), Birmingham (33°), and San Diego (32°).

Participants were asked to bring in all medications currently being administered so that the study team could cross-verify accuracy from the self-reported questionnaire. Medications tested for confounding have been implicated as potentially associated with falls in previous studies and included central nervous system medications (CNS), hypertensive medications, hypotensive medications and glucose medications. CNS medications included antidepressants, benzodiazepines, anticonvulsants, nonbarbituate sedative hypnotics and Zolpidem. Hypertensive medications included alpha-adrenergic blockers, beta blockers, calcium channel blockers, loop dieuretics, potassium-sparing diuretics and thiazide diuretics. Hypotensive medications included hypotensive agents-angiotensin II. Glucose medications included hypoglycemic agents and insulin.

History of falls was determined at the baseline self-administered questionnaire that asks participants, "During the past 12 months have you fallen and landed on the floor or ground, or fallen and hit an object like a table or chair". The Physical Activity Scale for the Elderly (PASE), a validated measure of physical activity level was administered at baseline visit and has been described elsewhere.[42] A variable was created for this analysis from the PASE questionnaire to measure physical activity outdoors that might impact vitamin D levels. The variable "Outdoor" was created

from three PASE questions asking participants about walking activity outside the house, lawn care and yard work, and outdoor gardening over the past seven days.

Assays were conducted to obtain total 25(OH)D levels, DBP concentration and albumin to be used in the calculation of bioavailable and free 25(OH)D levels. Additionally, these calculations of bioavailable and free 25(OH)D levels included information obtained through genotyping of participant samples.

Assay Measurements

Vials of fasting morning blood were collected from participants at baseline and the preparation of samples has been described previously.[43] In brief, serum was prepared immediately after phlebotomy and stored at -70°C in vials protected from light exposure. Until assays were performed, all samples remained frozen. Analysis of 25(OH)D levels were performed using mass spectrometry at the Mayo Clinic. Controls were included in every other assay run by using duplicate pooled serum and it was found that intra-assay coefficient of variation was 4.9% and inter-assay coefficient was 4.4%.[43] The minimum detectable limit was 4 ng/ml (10 nmol/L) for $25(OH)D_2$ and 2 ng/ml (5 nmol/L) for $25(OH)D_3$. Serum 25(OH)D has been cited as the most reliable measure of an individual's vitamin D stores.[44] Total 25(OH)D levels were calculated as the sum of $25(OH)D_2$ and $25ODH_3$ levels.

Assays on stored serum for DBP were completed at the Oregon Health and Science University Clinical and Translational Research Center lab (OCTRI) in December 2012 using the Quantikine Human Vitamin D Binding Protein immunoassay (R&D

Systems, Inc., Minneapolis, MN). One low and one high control were included in each vitamin D binding protein assay and multiple assays were conducted over a total of ten different days. As indicated in the Quantikin protocol, all samples were kept at room temperature before use. The inter-assay CV for the average of low and high controls was 2.08%. The intra-assay CV of high controls for DBP assay was 1.85% and for low controls was 2.27%.

Assays on baseline serum for albumin concentration were conducted at the Oregon Veterans Administration Clinical Lab using a Roche COBAS Integra 800 automated analyzer (Roche Diagnostics Corp., Indianapolis, IN). One control was included in each assay run and the analyzer was calibrated on a daily basis in the clinical laboratory. The detectable ranges for albumin were 0.2 g/dl to 6.0 g/dl and the inter-assay CV was 1.98%.

Genotyping was performed at Oregon Health and Science. TaqMan® SNP Genoytyping Assays were used to assess allele prevalence in our study population. Briefly, a GeneAmp® PCR System 9600 (Applied Biosystems, Foster City, CA) was used to amplify SNPs Rs4588 and Rs7041 from 10 pg of DNA using SNP Assay Mixes C__8278879 and C__3133594 (Applied Biosystems), respectively. Manufacturer-provided reaction constituents and thermo-cycling conditions (2min 50ºC, 10min 95 ºC, 50x 15s 95 ºC / 1min 60 ºC) were used in all cases. Allelic discrimination plots were generated in real-time using ABI PRISM® 7000 software (Applied Biosystems). Haplotypes were estimated using PHASE software for reconstructing haplotypes from population data.

Bioavailable 25(OH)D levels and Free 25(OH)D were estimated at the University of California Los Angeles using previously validated mathematical extra-cellular steady state models in the software Matlab by Rene Chun. These models determined bioavailable and free 25(OH)D levels by accounting for total 25(OH)D levels, genotypically determined binding affinity, DBP concentration and albumin concentration.[26]

At baseline, the physical function tests of ability to complete narrow walk course, rise from a chair without the use of arms, usual walking pace and grip strength were conducted. These physical function measures along with appendicular skeletal mass were examined as potential mediators in the relationship between bioavailable 25(OH)D and falls.

Narrow Walk

At baseline, participants were asked to complete a narrow walking course. Over three attempts, participants were asked to walk six meters without stepping outside of a 20-cm path. Stepping outside of the path or relying on an outside source such as a wall, chair or study staff member to maintain balance was considered a deviation. Participants with two or fewer deviations were considered successful and those with more than two were considered unsuccessful.

Chair Stand

Participants were asked to rise from a standard chair with their arms crossed over the course of five attempts. Time to complete five chair stands was used as a continuous variable with those unable to complete the task assigned the same time as the highest score for those able to complete the task.

Walking Pace

Walking pace was measured at baseline in meters/second on a standard six meter walking course. Walking pace was treated as a continuous variable with those unable to complete the measure assigned the lowest scoring value of participants successfully completing the measure. Additionally, we examined the average of the number of steps participants used during walking pace test one and two as a continuous variable.

Grip Strength

A JAMAR hydraulic hand-held dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) was used to measure grip strength. Two trials were performed for each hand and the maximum value achieved for either right or left grip was used as a continuous variable. Participants unable to complete the grip strength test were assigned the lowest value from those completing the task.

Appendicular Skeletal Mass

Appendicular skeletal mass was derived using dual energy X-ray absorptiometry (DEXA) at the baseline visit. All sites used the same model of DEXA machines (QDR 4500, Hologic Inc, Waltham, MA, USA) and all DEXA technicians were trained in standard operating procedures and certified. Cross-calibration of machines was performed at all sites prior to baseline visits and monitored by a central quality control lab. Machines were calibrated daily to assure reproducibility. Appendicular skeletal mass has been found to be highly correlated with skeletal muscle mass ($r=0.82$) and comprises the non-bone, non-fat muscle tissue of participant extremities.[45]

Prospective Falls

Falls were self-reported every four months by mailed postcard questionnaires during twelve months of follow-up, starting with the participant's baseline visit. Although follow-up information for falls was available beyond the first year, we chose to focus only on the initial year of follow-up since this reflected the time period with the most relevant proximity to the baseline assay of vitamin D levels. Postcards were sent to all active participants in July, November and March. Falls were examined in two ways, first as zero falls compared to one or more falls within the first year and then as 0-1 falls compared to 2 or more falls within the first year. Participants with missing fall information at any point during the first year were only included in the recurrent fall analyses if they had achieved two or more falls within the non-missing time points. Participants with no or one fall recorded during the first year but missing follow-up information were excluded from the recurrent falls analyses to avoid potential misclassification. In our cohort of 674, one participant was missing fall information at time point one, two participants died at time point two and one participant died at time point three.

Statistical Analysis

All analyses were conducted using SAS 9.2 and SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA). Our final cohort consisted of the 674 men within the MrOS study who had bioavailable 25(OH)D measures and fall information for the first year of follow-up. Serum bioavailable 25(OH)D levels were divided into quartiles. For categorical variables, descriptive statistics were calculated using chi-square tests if cells had an adequate number of observations or Fisher's exact test when cell sizes were less than five. For continuous variables, descriptive statistics

were calculated using one-way ANOVA for normally distributed variables and Kruskal-Wallis tests for non-normally distributed variables.

Log-binomial regression models were used to obtain relative risk (RR) estimates for each fall outcome in relation to bioavailable 25(OH)D quartile. We first conducted our analysis with the dependent variable as any falls in the first year of follow-up compared to no falls in the first year of follow-up. We then repeated the analysis with the dependent variable as two or more falls in the first year of follow-up compared to no or one fall in the first year of follow-up. Total 25(OH)D was then divided into quartiles and examined with the final models (lacking bioavailable 25(OH)D) to examine any associations with the dependent fall variable of interest.

In previous studies, non-Caucasian race, older age, higher BMI, winter months, and high latitude have all been associated with lower levels of total 25(OH)D concentration.[30] Given these associations, a priori covariate selection included race , age, BMI, season and latitude of clinic site. Age and BMI were entered as continuous variables. Season of blood draw was classified into fall, winter, spring and summer. Potential confounding of the association between falls and bioavailable 25(OH)D quartile was determined by a greater than ≥10% change in the RR estimate after entering in the potential confounding variable. Potential confounding variables included education, smoking history, alcohol history, history of falls, physical activity (PASE score), total intact PTH, total testosterone, hypertensive medication use, hypotensive medication use, glucose medication use, CNS medication use, vitamin D supplement use, self-rated health, outdoor activity level, history of dizziness, history of arthritis, history of stroke, history of cancer, history of angina, and history of Parkinson's disease. No confounders were found that resulted in a 10% or greater

change in RR estimates, therefore our final model only included adjustments for race, age, BMI, season of blood draw and latitude.

Potential mediators in our analysis included appendicular lean mass and the physical function variables of grip strength, ability to complete narrow walk test, walking pace test, chair stand test, and the average number of steps for the usual walking pace test. Grip strength was selected to test muscular strength; chair stand to test strength, coordination and balance; narrow walk to test balance and coordination; and walking pace to test neuromuscular function. Average number of steps for the usual walking pace test was examined because tripping represents the most frequent cause of falls in older populations and short steps indicative of shuffling have been associated with an increased risk of falling. [46, 47] We examined potential mediating variables in the relationship between bioavailable 25(OH)D and falls in two ways (Figure 2). The first mediation analysis involved two steps to determine if significant associations existed for both steps of regression analyses. Step one employed log-binomial regression to examine the adjusted RR estimate and p-value for the potential mediator with any falls as the dependent variable. Step two used the potential mediator as the dependent variable with bioavailable 25(OH)D as a continuous independent variable. Linear regression was used to examine all continuous potential mediators in step two to obtain beta estimates and p-values for the independent variable of bioavailable 25(OH)D. Log-binomial regression was used to examine the categorical narrow walk mediation variable to obtain RR estimates and p-values for the independent variable of bioavailable 25(OH)D. The second mediation analysis simply involved entering each potential mediating variable individually into the final log-binomial any falls model to examine attenuation in the RR estimates.

Results

Study Population Descriptives

Of the 674 men in the sample for this analysis, 26% were classified as Vitamin D deficient as defined by a serum total 25(OH)D level <50 nmol/L (20 ng/mL), 51% were classified as insufficient as defined by a total 25(OH)D between 50 nmol/L and <75 nmol/L, and 23% were classified as optimal as defined by a total 25(OH)D level ≥75 nmol/L (30 ng/mL). The mean (±sd) for bioavailable 25(OH)D was 12.98 (5.65) nmol/L and for total 25(OH)D was 62.2 (±19.9) nmol/L. Those in the lowest bioavailable 25(OH)D quartile had statistically significant lower values for total 25(OH)D level and PASE score and significantly higher levels of DBP (Table 1). The correlation between bioavailable 25(OH)D and total 25(OH)D was r=0.55, PASE score r=0.14, and DBP r=-0.49. The lowest quartile for bioavailable 25(OH)D had slower paces on the narrow walk test, a higher number of steps for the usual walking pace test, and lower grip strength measures. Those in the highest quartile for bioavailable 25(OH)D were least likely to be Non-Hispanic white. However, for total 25(OH)D quartile, those in the lowest quartile were least likely to be Non-Hispanic white.

Prospective Falls Analysis

During the first year of follow-up, a fall was reported by 195 men (29%). The lowest quartile of bioavailable 25(OH)D, quartile 1, had the highest proportion of falls (35%) and quartile 3 reported the lowest proportion of falls (20%) (Table 2). Using the highest quartile, quartile 4, as referent showed no significantly lowered risk of falls

for those in this referent compared to those any other but did show an elevated risk compared to those in quartile 3, RR=0.65 (95% CI: 0.45 to 0.95).

The number of participants experiencing two or more falls during the first year of follow-up was much lower than those reporting any falls (n=106, 16%), but showed a similar pattern as single falls analysis. The effective sample for recurrent falls analyses was 670 men due to incomplete follow-up information for 4 individuals who were missing information for at least one other point due to lack of response (n=1) or death (n=3) and had less than two falls recorded at the other time points. Like the single fall analysis, the highest proportion of recurrent falls was in quartile 1 (20.8%) and the lowest proportion of recurrent falls was found in quartile 3 (12.5%). Compared to the highest quartile of bioavailable 25(OH)D, those in the 3rd quartile had a non-statistically significant RR of 0.81 for recurrent falls (95% CI: 0.47 to 1.39).

No statistically significant associations were observed for the single falls analysis or the recurrent falls analysis with total 25(OH)D quartiles or total 25(OH)D categories (deficient, insufficient, optimal). When looking at the single falls analyses there was no indication of an effect of Total 25(OH)D. However, the confidence intervals for recurrent falls were suggestive of a possible effect for the lowest quartile of Total 25(OH)D compared to the highest, RR=1.37 (95% CI: 0.82 to 2.28) as well as for those classified as vitamin D deficient compared to optimal, RR=1.45 (95% CI: 0.86 to 2.46).

Mediation Analyses

Attenuation in RR estimates was minor after adding the potential mediating variables individually into the final model, indicating little effect of these variables in the observed relationship between falls and bioavailable vitamin D (Table 3). We were therefore unable to identify any mediators among those examined in the relationship between falls and bioavailable 25(OH)D.

Potential mediation was also examined by building separate models with the potential mediator as the independent variable in one model and the dependent variable in the other model to determine if significant associations were seen for both (Tables 4-6). Mediators which showed statistically significant relationships for both models included grip strength and average number of steps for usual walk speed trials. For every increase in number of steps, the RR of falls increased by 1.1 times, $p<0.001$. When examining number of steps as the outcome variable, every unit increase in bioavailable 25(OH)D was associated with a 0.03 decrease in the average number of steps, $p=0.01$. For grip strength, the RR of falls decreased by 0.98 times with each increase in grip strength, $p=0.02$. When examining grip strength as the outcome variable, every unit increase in bioavailable 25(OH)D was associated with a 0.12 increase in grip strength, $p=0.04$. However, given the lack of attenuation seen in the previous mediation analysis when mediators were entered into the final bioavailable quartile models, these results do not provide adequate evidence of mediation effects.

Spline Analyses

Based on a number of previous studies which found non-linear patterns for vitamin D and a variety of health outcomes, coupled with our finding of a reduced relative risk for the third quartile, we further examined the linearity of the vitamin D metabolites

with falls through the use of splines. Spline analyses examining any falls with Total 25(OH)D and separately with bioavailable 25(OH)D as continuous variables suggested non-linear patterns for both measures. Spline models were adjusted for age, BMI, race, season of blood draw and latitude. For Total 25(OH)D, nonlinearity was of marginal significance (p=0.06) and suggested a U-shaped curve with the probability of falls decreasing steadily for serum concentrations up to approximately 55 nmol/L and then increasing sharply with 25(OH)D serum concentrations exceeding approximately 100 nmol/L (Figure 3). In this sample, 26 men had serum concentrations at or above 100 nmol/L. We considered the possibility that this pattern might be the result of increased total 25(OH)D serum concentrations due to vitamin D supplement use among those at greater risk of falls so we reran the spline analysis adjusting for vitamin D supplementation and saw a similar pattern toward non-linearity (Figure 4).

Conducting spline analyses on bioavailable 25(OH)D again showed marginal significance for nonlinearity (p=0.06) but the trend appeared to be cubic (Figure 5). The curve suggested an increase in the probability of falls between bioavailable 25(OH)D levels of 15 nmol/L and 20 nmol/L with a decrease in probability with increasing concentration for levels above and below this range. Adjusting for vitamin D supplement use did not alter this relationship (Figure 6). The cubic nature of this relationship with falls was further examined by adding a centered cubic term for bioavailable 25(OH)D to a model adjusted for age, BMI, race, season of blood draw and latitude. Both squared and cubic bioavailable 25(OH)D terms were statistically significant in this model (p=0.02 and p=0.04, respectively).

Discussion

In this study, we investigated the relationship between bioavailable 25(OH)D levels and falls in men 65 years and older. We also examined physical function and appendicular skeletal mass as potential mediators in this relationship. In the random sample of ambulatory community-dwelling men, those in the highest quartile of bioavailable 25(OH)D did not have any significantly lower risk of falls compared to any other quartile. A statistically significant reduction in risk was found for the third quartile of bioavailable compared to the highest quartile (quartile 4) with those in the third having a 34% reduction in fall risk. When comparing recurrent fallers, no statistically significant association was found however power calculations revealed that we only had the power to detect a RR of 1.5 or greater between the highest and lowest quartiles for recurrent falls. The pattern for recurrent fallers was similar to the pattern for any falls with the lowest proportion of recurrent falls occurring in the third quartile of bioavailable 25(OH)D and the highest proportion occurring in the lowest bioavailable 25(OH)D quartile.

Non-linear relationships with serum 25(OH)D have been reported in previous studies investigating various health outcomes. Most relevant to this study is a meta-analysis conducted by the IOM committee which found that a U-shaped curve best described the relationship between total serum 25(OH)D levels and risk of falls in a previous meta-analysis conducted by Bischoff-Ferrari et. al in 2009.[14] In this meta-analysis, the IOM fit a random effects meta-regression model for the log(RR) of any falls with total 25(OH)D serum concentration or the daily dose of vitamin D supplementation

and although they found no evidence of a linear relationship for either variable, entering in a quadratic term into the models suggested a U-shape curve.

A number of studies have identified non-linear relationships of vitamin D levels with cancer, cardiovascular events and all-cause mortality.[6, 48-53] In a 2012 study by de Koning et al., a non-linear relationship was found between total 25(OH)D levels and hip fracture risk.[54] In this study, an inverse association between total 25(OH)D and prospective hip fracture was only significant for levels less than 70 nmol/L. Further in a longitudinal case-control sample of Nordic men, levels of 25(OH)D at or below 19 nmol/L were associated with an increased risk of prostate cancer as were levels at or above 80 nmol/L.[55]

Given these past non-linear associations with total 25(OH)D and the finding that compared to the highest quartile of bioavailable 25(OH)D, those in the next highest quartile had a 0.65 RR of a fall we employed spine analyses to explore the possibility of a non-linear relationship between the vitamin D metabolites and falls. This analysis revealed a cubic shape with risk of falls showing an inverse association with bioavailable 25(OH)D, then a positive association, followed by a return to an inverse association. As we can find no biologically plausible explanation for such a relationship, this association may be the result of residual confounding or bias, random variation or chance in the random sample. However, the right and left sides of the bioavailable 25(OH)D spline mirror each other well and would be suggestive of a potential linear relationship other than the dip occurring in the middle of the spline. As far as we are aware, this is the first study to examine fall risk with bioavailable 25(OH)D. Future studies should further investigate this relationship to determine if a

biologically plausible non-linear or linear pattern exists or if falls and bioavailable 25(OH)D are not associated.

Although statistically significant relationships were found during mediation analysis for the variables of grip strength and average number of steps for usual walking pace with both falls and with bioavailable 25(OH)D, entering these variables into our final models did not substantially change the RR estimates for the statistically significant quartile of bioavailable 25(OH)D.

Our study had a number of strengths. The cohort is well-characterized with almost complete follow-up and represents the age group most likely to be affected by both falls and vitamin D deficiency. Further, this study represents to the best of our knowledge the first examination of bioavailable vitamin D with fall risk. Additionally, unlike many previous studies on fall risk and vitamin D our study was able to examine and eliminate outdoor activity as a potential confounding variable in the relationship. Finally, the prospective nature of our study allowed us to assess bioavailable 25(OH)D levels at baseline and then follow participants forward for the first year following blood draw to determine how baseline concentrations were associated with future falls.

However there are important limitations to our study which should be considered. First, is the small sample size that limited our ability to assess how bioavailable vitamin D affected recurrent falls. An additional potential limitation is that to our knowledge, there are currently no clinically relevant guidelines on what constitutes inadequate or optimal levels of bioavailable 25(OH)D. Therefore, it is possible that

the participants in this cohort had a higher or lower range of bioavailable 25(OH)D levels that limited both the observed effects as well as the generalizability to the general population of seniors. Further, the MrOS study cohort comprises relatively healthy older men which might limit the variation in bioavailable 25(OH)D or falls to such a degree as to inadequately capture the true nature of the association. However, the proportion of falls in our analysis was consistent with the approximate 30% annual prevalence of falls in those over 65 years reported elsewhere.[56-58] Another potential limitation involves the small numbers of other race/ethnicity men in the MrOS cohort which resulted in our inability to divide race/ethnicity into categories more specific than Non-Hispanic white and other race/ethnicity to determine how race and bioavailable vitamin D status might potentially interact. Finally, the use of self-report for falls opens the possibility that participants may inaccurately report falls during the study period. However the MrOS study has been carefully designed to increase the accuracy of prospective fall reports by utilizing a tri-yearly fall report. Additionally, any miscategorization of falls is likely to be non-differential since participants were unaware of their bioavailable 25(OH)D status.

Declining mortality rates and advances in medical technology indicate that more people will be surviving to advanced ages than in previous decades. By 2040, 20% of the adult population will be over 65 years of age and the population over 85 years is projected to more than triple from the 2000 percentage.[59] Falls represent a significant contributing factor to disability and impairment in aging populations. Given the high financial and emotional costs associated with declining independence in old age, identification of new factors that prevent disability and falls are crucial to reducing future burden on the health care system. As the baby boomer generation

ages and those over 65 years of age represent a larger proportion of the population, the importance of fall avoidance with advancing age will become even more critical. Findings from this study have the potential to serve as an important first step in identifying levels of bioavailable vitamin D that may be associated fall risk. Additionally, research into the effects of bioavailable vitamin D on health outcomes is inadequately represented in the literature therefore this study contributes to a developing body of knowledge on the use of bioavailable vitamin D concentration as opposed to total vitamin D concentration in the examination of health outcome associations.

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Tables and Figures

Table 1: Characteristics of the analytic cohort of 674 men aged 65 years and older by Bioavailable 25(OHD) quartile

	Bioavailable 25(OH)D Quartile				
	Q1 (n=169)	Q2 (n=168)	Q3 (n=168)	Q4 (n=169)	p-value
Bioavailable 25(OH)D, nmol/L, mean, (SD)	7.05 (1.69)	10.59 (0.81)	13.66 (1.02)	20.65 (4.76)	**<0.001***
Total 25(OH)D, nmol/L, mean, (SD)	45.06 (15.82)	62.43 (14.40)	67.70 (17.66)	73.64 (19.02)	**<0.0001***
Vitamin D Binding Protein µmol/L, mean, (SD)	295.28 (88.12)	289.57 (75.48)	242.23 (72.45)	179.08 (74.31)	**<0.0001***
Haplotypes					
1F1F, n, (%)	6 (3.55%)	3 (1.79%)	4 (2.38%)	15 (8.88%)	**<0.0001***
1F1S, n, (%)	39 (23.08%)	28 (16.67%)	34 (20.24%)	8 (4.73%)	
1F2, n, (%)	5 (2.96%)	8 (4.76%)	14 (8.33%)	33 (19.53%)	
1S1S, n, (%)	94 (55.62%)	83 (49.40%)	30 (17.86%)	24 (14.20%)	
1S2, n, (%)	25 (14.79%)	44 (26.19%)	83 (49.40%)	51 (30.18%)	
22, n, (%)	0 (0%)	2 (1.19%)	3 (1.79%)	38 (22.49%)	
Demographic Factors					
Age in years, mean, (SD)	75.06 (6.64)	74.2 (5.94)	73.78 (5.98)	73.49 (5.32)	0.2
Other Race/Ethnicity, n (%)	16 (9.47%)	3 (1.79%)	13 (7.74%)	23 (13.61%)	**<0.001***
College Degree, n (%)	90 (53.25%)	80 (47.62%)	96 (57.14%)	84 (49.7%)	0.32
Lifestyle and Health					
Self Rated Health: Fair-Very Poor, n (%)	33 (19.53%)	20 (11.90%)	26 (15.57%)	23 (13.61%)	0.43
Smoking status (Former or Current), n, (%)	107 (63.31%)	93 (55.36%)	108 (64.29%)	91 (53.85%)	0.11
Alcoholic drinks per week, mean, (SD)	4.62 (7.27)	3.59 (5.64)	4.54 (7.12)	4.23 (5.49)	0.48
Medication Use, n, (%)					
CNS Medications	21 (12.43%)	23 (13.69%)	13 (7.74%)	16 (9.47%)	0.28
Glucose	20 (11.83%)	12 (7.14%)	12 (7.14%)	21 (12.43%)	0.18
Hypertensive Medication	90 (53.25%)	78 (46.43%)	87 (51.79%)	91 (53.85%)	0.51
Hypotensive Medication	6 (3.77%)	10 (6.41%)	11 (6.75%)	8 (4.79%)	0.61
Total Number of Medications, mean, (SD)	4.92 (4.28)	3.94 (3.55)	4.21 (3.71)	4.26 (3.35)	0.11
Vitamin D supplement use, n, (%)	12 (7.36%)	25 (15.34%)	24 (15.38%)	24 (14.72%)	0.09
Appendicular skel lean , kg, mean (SD)	23.77 (3.82)	24.22 (3.30)	24.55 (3.45)	24.12 (2.94)	0.17
BMI, mean, (SD)	27.62 (4.29)	27.39 (3.73)	27.57 (3.52)	26.90 (3.24)	0.27
Baseline history of falls, n, (%)	41 (24.26%)	38 (22.62%)	33 (19.64%)	35 (20.71%)	0.74
Physical Function and Activity					
PASE score, mean, (SD)	129.94 (63.96)	146.88 (60.79)	155.44 (70.08)	153.61 (65.91)	**0.0021***
Seconds to complete 5 chair stands	12.94 (6.11)	11.50 (4.26)	11.70 (4.21)	11.89 (4.75)	0.32
Walking Pace, m/s, mean, (SD)	1.19 (0.27)	1.23 (0.25)	1.25 (0.23)	1.26 (0.23)	0.13
Average no. steps for usual walking pace, mean (SD)	10.10 (2.15)	9.70 (1.93)	9.46 (1.40)	9.46 (1.54)	**0.01***
Unable to Complete Narrow Walk Test, n, (%)	38 (23.17%)	29 (17.90%)	26 (15.76%)	24 (14.37%)	0.17
Narrow Walk Pace m/s, mean (SD)	0.98 (0.39)	1.04 (0.36)	1.09 (0.33)	1.10 (0.37)	**0.01***
Grip Strength, kg, mean (SD)	38.91 (8.80)	41.17 (8.51)	41.41 (8.46)	41.45 (9.32)	**0.01***

†Quartile 1 is the lowest quartile for Bioavailable 25(OH)D level

*Significant at p-value of 0.05

Table 2: Vitamin D and falls in the first year of follow-up, MrOS cohort

	Range (nmol/L)	Any Falls, n (%)	**RR* (95% CI)**	**p-value**	2+ Falls, n (%)	**RR* (95% CI)**	**p-value**
Bioavailable 25(OH)D Quartile							
1	1.87-9.17	59 (35%)	**1.09 (0.80 to 1.50)**	0.57	35 (20.8%)	**1.21 (0.75 to 1.97)**	0.43
2	9.21-12.07	51 (30%)	**0.94 (0.68 to 1.30)**	0.73	26 (15.6%)	**0.98 (0.59 to 1.61)**	0.93
3	12.08-15.67	34 (20%)	**0.65 (0.45 to 0.95)**	0.03	21 (12.5%)	**0.81 (0.47 to 1.39)**	0.44
4	15.75-43.19	51 (30%)	Ref	Ref	24 (14.4%)	Ref	Ref
Total 25(OH)D Quartile							
1	7.81-49.57	58 (34%)	**1.11 (0.80 to 1.54)**	0.50	36 (21.4%)	**1.37 (0.82 to 2.28)**	0.24
2	49.67-61.90	40 (23%)	**0.79 (0.55 to 1.13)**	0.20	24 (14.0%)	**1.02 (0.60 to 1.72)**	0.89
3	61.93-73.88	47 (28%)	**0.92 (0.66 to 1.28)**	0.80	24 (14.5%)	**1.00 (0.60 to 1.68)**	0.89
4	73.93-139.28	50 (30%)	Ref		22 (13.2%)	Ref	Ref
Total 25(OH)D Category							
Deficient	7.81-49.92	60 (34.1%)	**1.17 (0.83 to 1.64)**	0.36	37 (21.1%)	**1.45 (0.86 to 2.46)**	0.17
Insufficient	50.04-74.93	91 (26.6%)	**0.93 (0.69 to 1.26)**	0.65	50 (14.7%)	**1.11 (0.68 to 1.81)**	0.66
Optimal	75.13-139.28	44 (28.2%)	**Ref**	Ref	19 (12.3%)	**Ref**	Ref

*Adjusted for race, age, BMI, season of blood draw and latitude
†Deficient defined as <20 ng/mL (50 nmol/L)
††Optimal defined as ≥30 ng/mL (75 nmol/L)

Table 3: Change in Quartile RR estimates when adding potential mediating variable into final bioavailable 25(OH)D any fall model

Variable	RR Q1 (% Change)	RR Q2 (% Change)	RR Q3 (% Change)
Base Model	**1.09 (ref)**	**0.94 (ref)**	**0.65 (ref)**
Grip Strength, kg	1.07 (2%)	0.93 (1%)	0.64 (1%)
Average # of Steps	1.06 (3%)	0.91 (3%)	0.66 (1%)
Narrow Walk	1.05 (4%)	0.92 (2%)	0.62 (3%)
Walking Pace, m/s	1.04 (5%)	0.91 (3%)	0.65 (0%)
Chair Stands, s	1.07 (2%)	0.95 (1%)	0.67 (2%)
Appendicular Skeletal Lean, kg	1.07 (2%)	0.93 (1%)	0.65 (1%)

Table 4: Model with any fall as the dependent variable and potential mediators as independent variable

Model With Any Fall as outcome variable

Independent Variable	RR	p-value
Grip Strength, kg	0.98	0.016*
Average # of Steps	1.1	<.0001*
Narrow Walk	1.5	0.005*
Walking Pace, m/s	0.32	<.0001*
Chair Stands, s	1.03	0.001*
Appendicular Skeletal Lean, kg	0.98	0.91

*All models adjusted for age, BMI, race, latitude

Table 5: Linear Regression Model with Bio25(OH)D as independent variable and potential mediators as dependent variable

Model with Bioavailable 25(OH)D as continuous independent variable

Dependent Variable	Beta	p-value
Grip Strength, kg	0.119	0.04*
Average # of Steps	-0.027	0.01*
Walking Pace, m/s	0.003	0.10
Chair Stands, s	-0.036	0.28
Appendicular Skeletal Lean, kg	0.014	0.44

All models adjusted for age, BMI, race, latitude, season

Table 6: Log-Binomial Regression Model with Bio25(OH)D as independent variable and ability to complete Narrow Walk test as dependent variable

Log-Binomial Regression model including Bio25(OH)D as independent variable

Dependent Variable	RR	p-value
Narrow Walk	0.997	0.21

Adjusted for age, BMI, race, latitude, season

Figure 1: Flowchart of inclusion exclusion criteria of Bioavailable 25(OH)D cohort from main analytic cohort

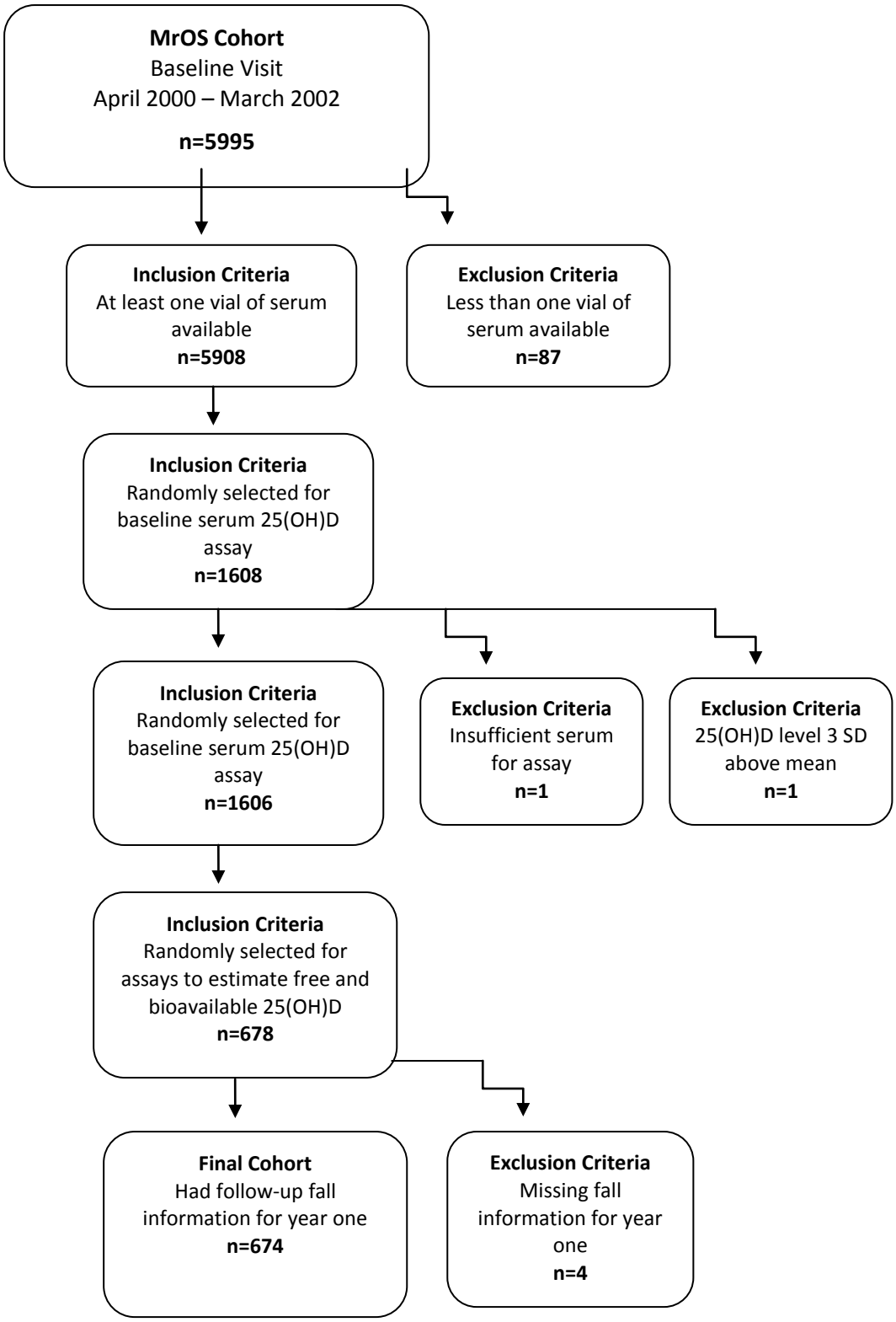


Figure 2 – Conceptual models relating bioavailable vitamin D to risk of falls with potential intermediate variables of physical function and skeletal lean mass

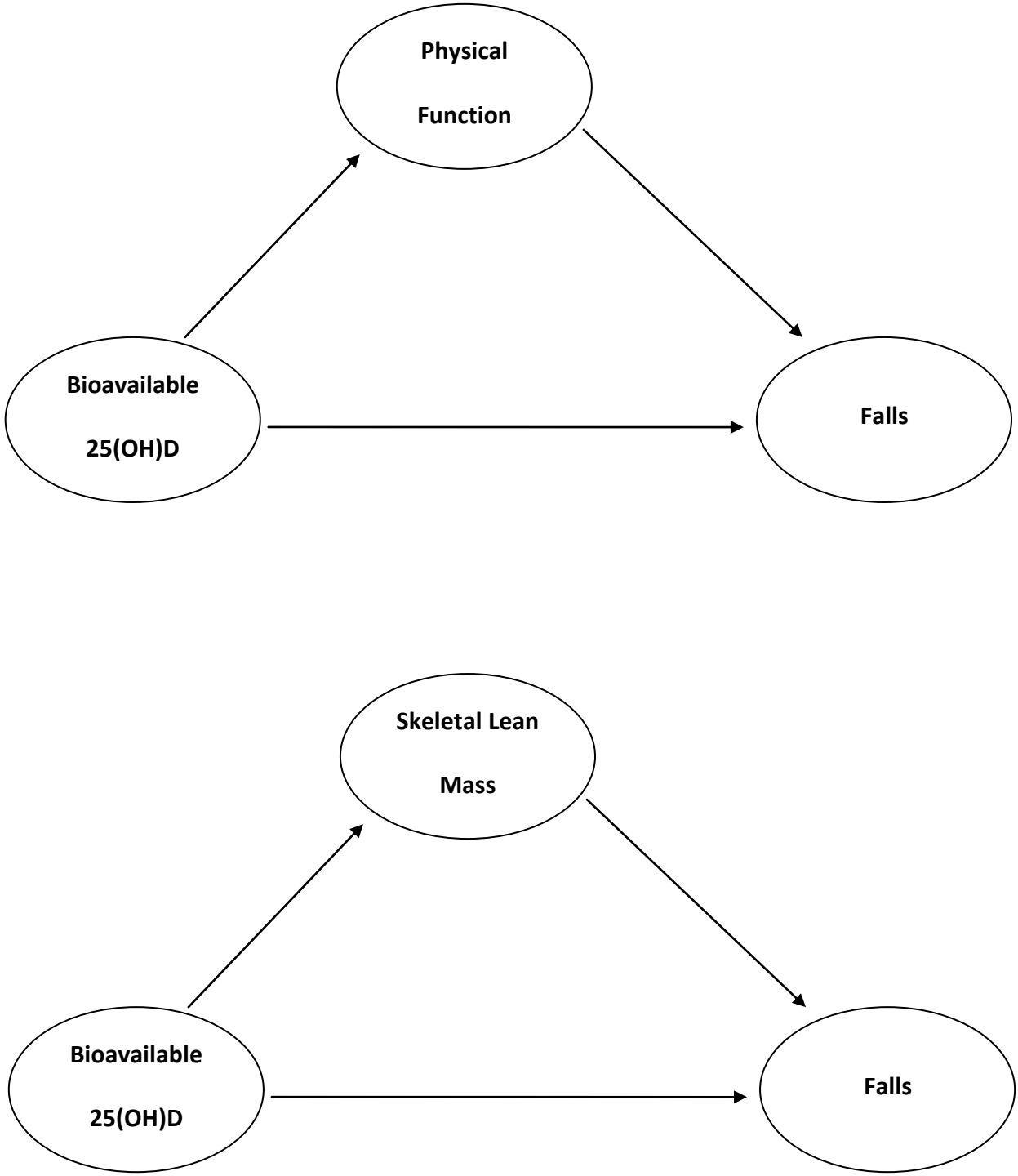
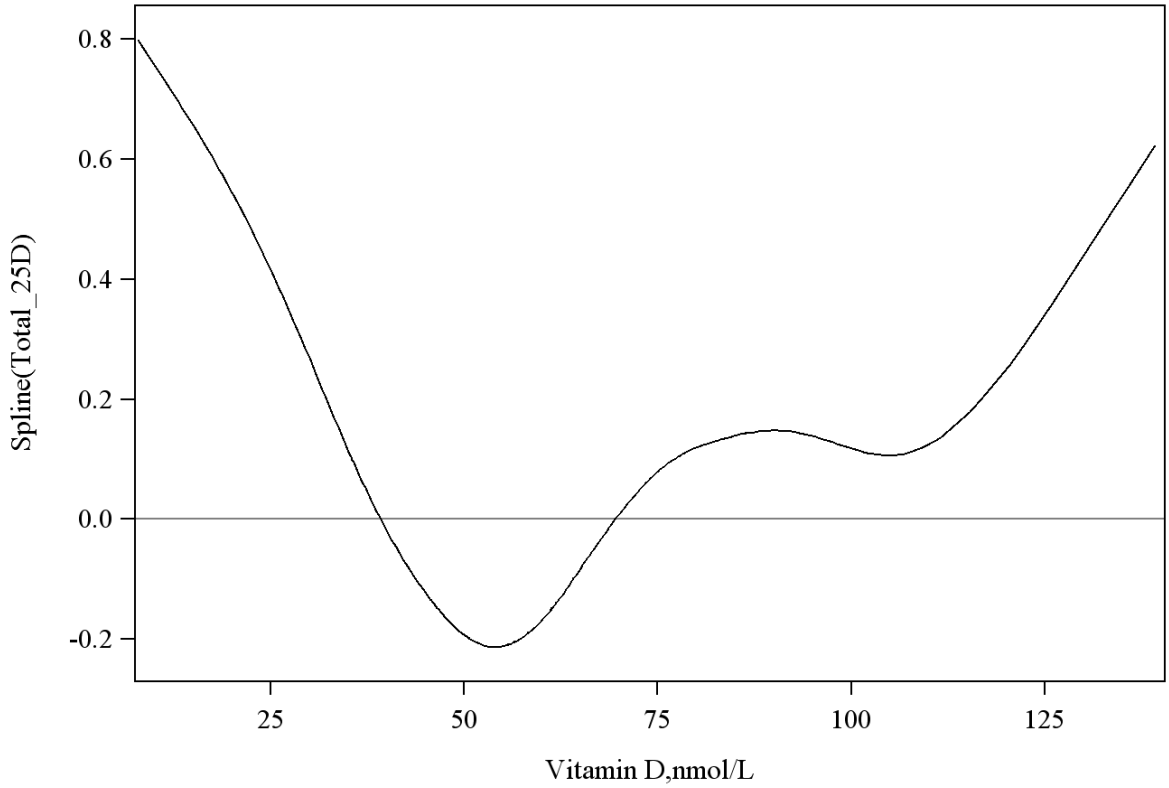
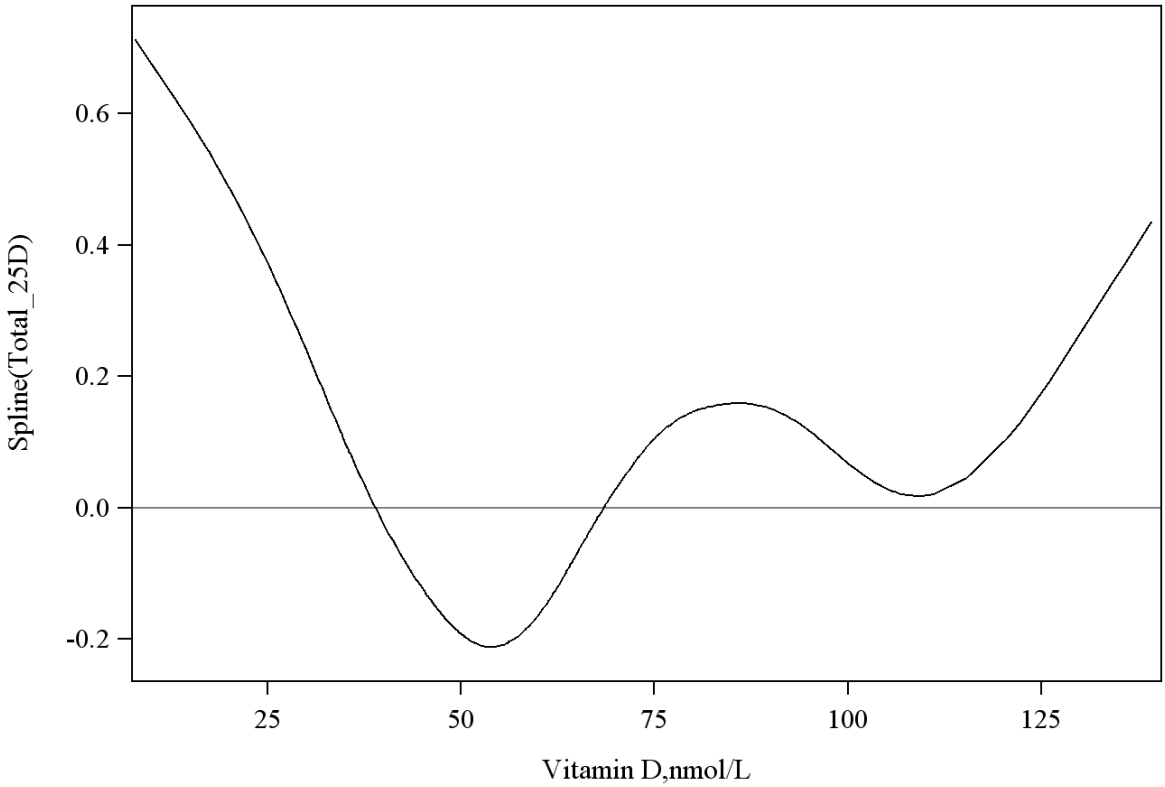


Figure 3: Spline analysis of Total 25(OH)D and any falls



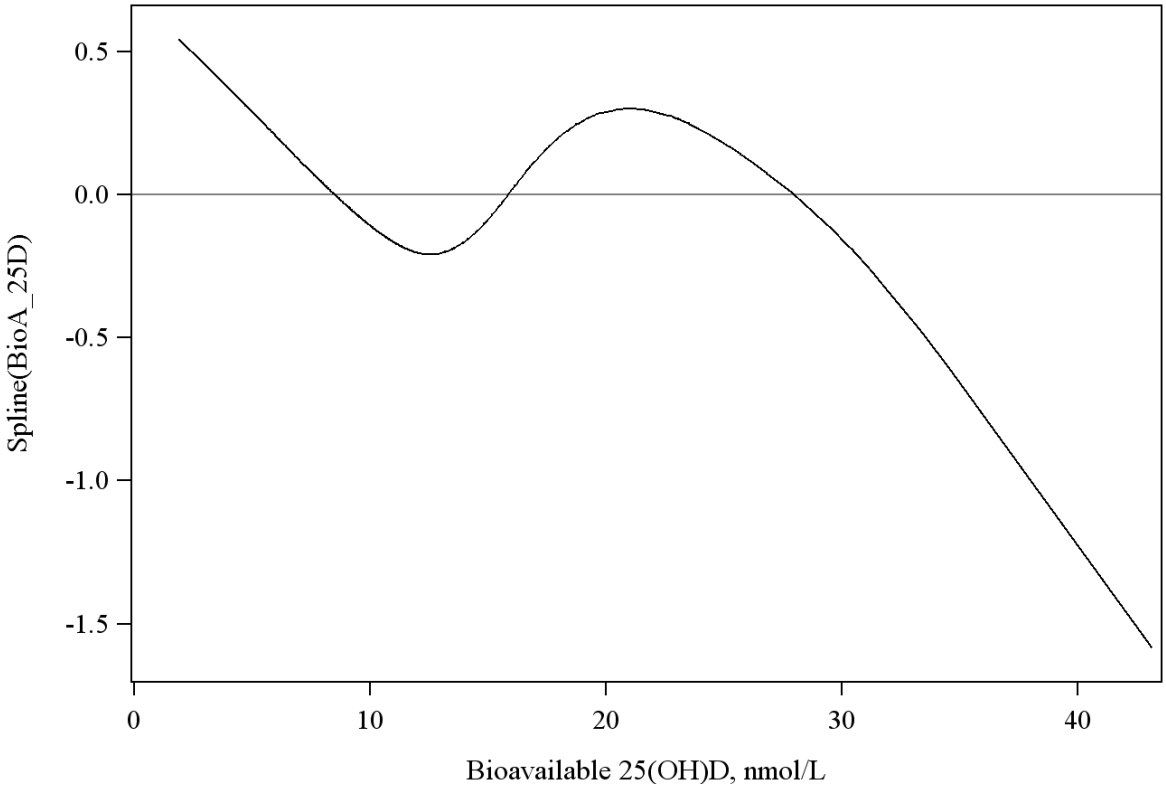
*Adjusted for race, age, BMI, season of blood draw and latitude

Figure 4: Spline analysis of Total 25(OH)D and any falls



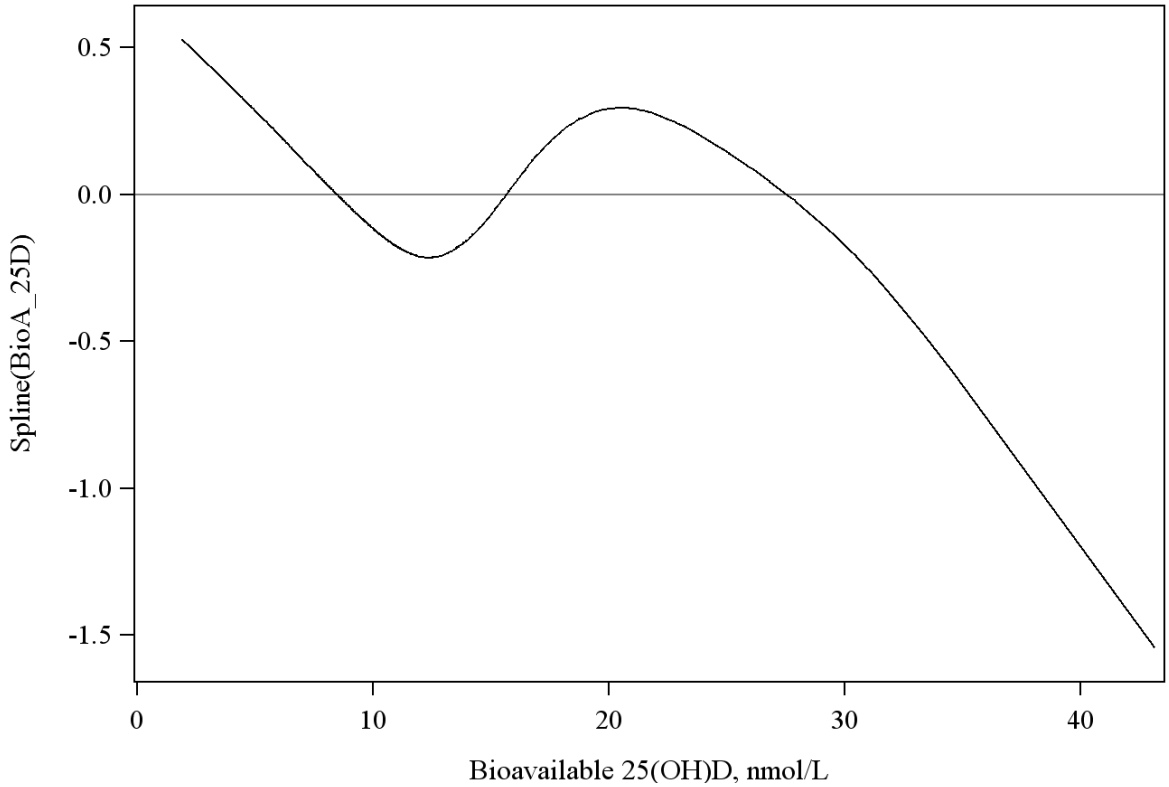
*Adjusted for race, age, BMI, season of blood draw, latitude and vitamin D supplement use

Figure 5: Spline analysis of Bioavailable 25(OH)D and any falls



*Adjusted for race, age, BMI, season of blood draw and latitude

Figure 6: Spline analysis of Bioavailable 25(OH)D and any falls



*Adjusted for race, age, BMI, season of blood draw, latitude and vitamin D supplement use

Table 7: Bioavailable 25(OH)D deciles and any falls in the first year of follow-up, MrOS cohort

Bioavailable 25(OH)D Decile	N	Range (nmol/L)	Any Falls, n (%)	**RR* (95% CI)**	**p-value**	**RR¥ (95% CI)**	**p-value**
1	67	1.87-6.60	28 (42%)	**1.78 (1.03 to 3.05)**	0.04	**1.65 (0.96 to 2.85)**	0.07
2	67	6.64-8.63	22 (33%)	**1.45 (0.81 to 2.60)**	0.21	**1.38 (0.77 to 2.48)**	0.28
3	68	8.64-9.75	22 (32%)	**1.40 (0.79 to 2.48)**	0.24	**1.32 (0.74 to 2.32)**	0.35
4	68	9.80-10.90	18 (26%)	**1.14 (0.63 to 2.09)**	0.66	**1.06 (0.58 to 1.94)**	0.84
5	67	10.92-12.08	20 (30%)	**1.32 (0.74 to 2.36)**	0.35	**1.22 (0.68 to 2.18)**	0.51
6	67	12.09-13.33	13 (19%)	**0.85 (0.44 to 1.64)**	0.63	**0.79 (0.40 to 1.54)**	0.48
7	67	13.36-14.65	15 (22%)	**0.99 (0.53 to 1.86)**	0.98	**0.95 (0.50 to 1.78)**	0.87
8	69	14.66-16.75	17 (25%)	**1.06 (0.57 to 1.97)**	0.86	**1.02 (0.55 to 1.89)**	0.96
9	67	16.76-20.52	25 (37%)	**1.65 (0.96 to 2.84)**	0.07	**1.58 (0.92 to 2.73)**	0.10
10	67	20.53-43.14	15 (22%)	**Ref**	Ref	**Ref**	Ref

*Adjusted for age and season

¥ Adjusted for age, season, BMI, race, latitude

Appendix

Description of Variables

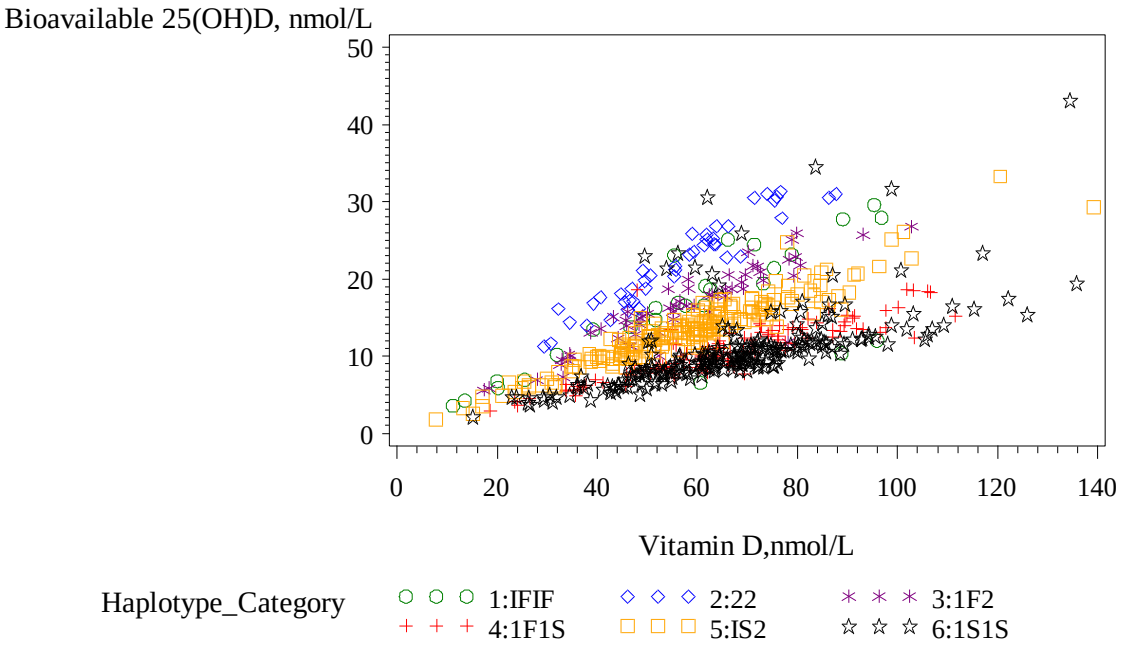
Variable	Measurement	Description
Bioavailable 25(OH)D	Morning fasting serum at baseline (Liquid chromatography/ Mass Spectrometry Assay) Calculated at UCLA using Haplotype, Total 25(OH)D levels and DBP information	Quartiles (nmol/L)
Any Falls	Tri-Annual Self-Report by postcard	Yes/No
2+ Falls	Generated from 1st three time periods of Tri-Annual Self-Report by postcard	Yes/No
Bioavailable 25(OH)D	Used only in Mediation Analysis	Continuous (nmol/L)
Total 25(OH)D	Morning fasting serum at baseline (Liquid chromatography/ Mass Spectrometry Assay)	Quartiles (nmol/L)
Vitamin D Binding Protein	Morning fasting serum at baseline (Liquid chromatography/ Mass Spectrometry Assay)	Continuous (µmol/L)
Vitamin D Binding Protein	Morning fasting serum at baseline (Liquid chromatography/ Mass Spectrometry Assay)	Quartiles (nmol/L)
Haplotype	GC Haplotype using variable GENOTYPE	1F1F, 1F1S, 1F2, 1S1S, 1S2, 22
Age	Verified age at enrollment	Continuous (years)
Race	Self-Report	Non-Hispanic white/Other race/ethnicity
BMI	Calculated using height and weight measurements	kg/m^2
Season	Generated from month of baseline visit	Winter (Dec-Feb), Spring (Mar-May), Summer (June-Aug), Fall (Sept-Nov)
Latitude	Latitude of clinic visit	High (Portland 45°, Minneapolis 44°, Pittsburgh 40°) Low (Palo Alto 37°, Birmingham 33°, San Diego 32°)

Education	Generated from self-report education variable GIEDUC	College Degree, Less than a college degree
Self Rated health	Generated from Self-reported QLHEALTH question "Compared to other people your won age, how would you rate your health?"	Excellent, Good, Fair-Very Poor
Smoking Status	Self-Report	Never, Former or Current
Alcohol Use	Self-Report number of drinks per week	Continuous
CNS Medications	Clinic Interview at Baseline generated from M1ADEPR (Antidepressant), M1BENZO (Benzodiazepine), M1NBAC (Anticonvulsant), M1NBANX (Nonbarbituate Sedative Hypnotic), M1ZOLP (Zolpidem)	Yes/No
Hypertensive Medications	Clinic Interview at Baseline generated from M1ALPHA (Alpha-Adrenergic Blocker), M1BETA (Beta Blocker), M1CABLOK (Calcium Channel Blocker), M1DILOOP (Loop Diuretic), M1DIPOTA (Potassium-Sparing Diuretic), M1DUITHX (Thiazide Diuretic)	Yes/No
Hypotensive Medication	Clinic Interview at Baseline M1ARB (Hypotensive Agents-Angiotensin II)	Yes/No
Glucose Medication	Clinic Interview at Baseline generated from M1HYPOG (Hypoglycemic Agents) and M1INSULIN (Insulin Use)	Yes/No
Vitamin D Supplement Use	Self-Report at Baseline generated from DTVITDF	Yes/No
Appendicular Skeletal Muscle	Dual energy X-Ray absorptiometry (DXA)	Continuous (kg)
Total Testosterone	Gas chromatographic-negative ionization tandem mass spectrometry and liquid chromatographic electrospray tandem mass spectrometry bioanalytical method. Average of 2 aliquouts from each participant	Continuous (ng/dl)
Total Intact PTH	Immunoradiometric Assay	Continuous (pmol/L)

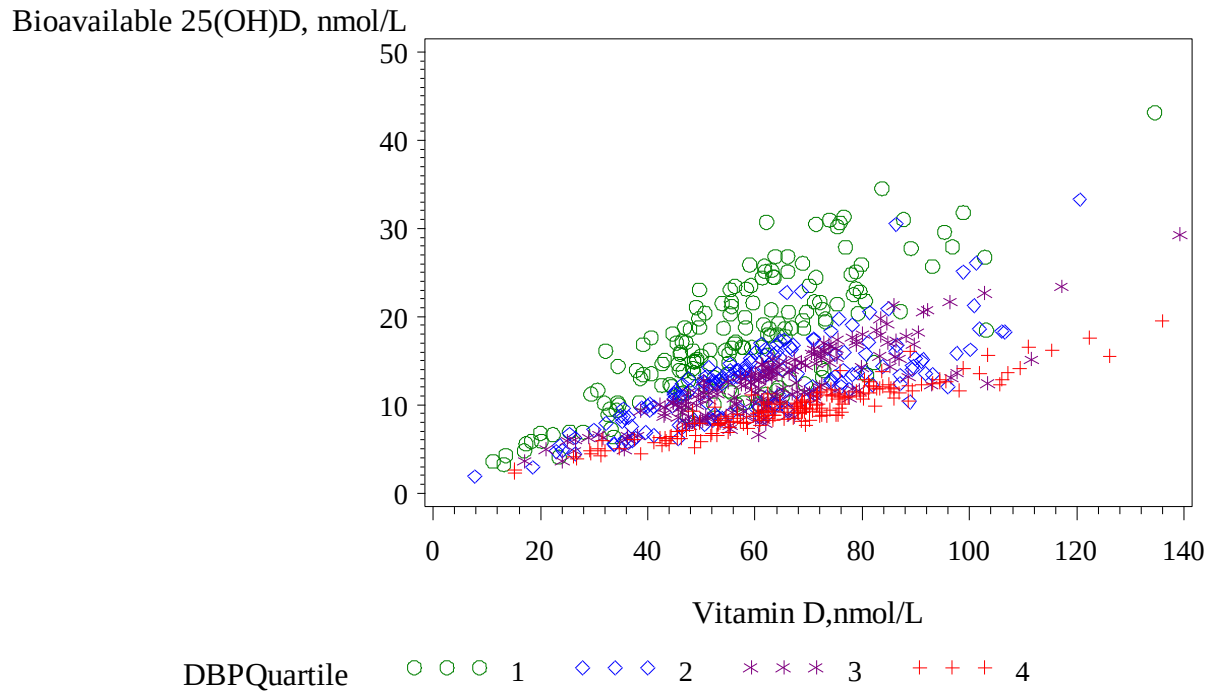
Baseline History of Falls	Self-report at baseline	Yes/No
PASE Score	Calculated Physical Activity Scale for the Elderly self-report score	Continuous
Outdoor Activity	Generated from self-report on PASE questionnaire of walking activity outside the house, lawn care and yard work, and outdoor gardening	Yes/No
Seconds to complete 5 chair stands	Time to complete 5 chair stands at baseline	Continuous (seconds)
Walking Pace	Pace in meters/second of best time of trials to complete a standard six meter walking course	Continuous (m/s)
Average Number of Steps for Walking Pace	Average of the number of steps taken during the two trials of Walking Pace. Generated from number of steps on time 1 and time 2 divided by 2.	Continuous
Narrow Walk Pace	Pace in meters/second of best time of three narrow walk trials in which participant was instructed to walk 6 meters without stepping outside of the 20-cm path	Continuous (m/s)
Grip Strength	Best grip strength achieved from 2 trials completed for each hand using a JAMAR Handheld dynamometer	Continuous (kg)
History of Arthritis	Self-Report at Baseline	Yes/No
History of Stroke	Self-Report at Baseline	Yes/No
History of Dizziness	Self-Report at Baseline	Yes/No
History of Parkinsons	Self-Report at Baseline	Yes/No
History of Angina	Self-Report at Baseline	Yes/No
History of Cancer	Self-Report at Baseline	Yes/No

Scatter plot of Total 25(OH)D and Bioavailable 25(OH)D by Haplotype

Total 25(OH)D and BioA25(OH)D by Haplotype

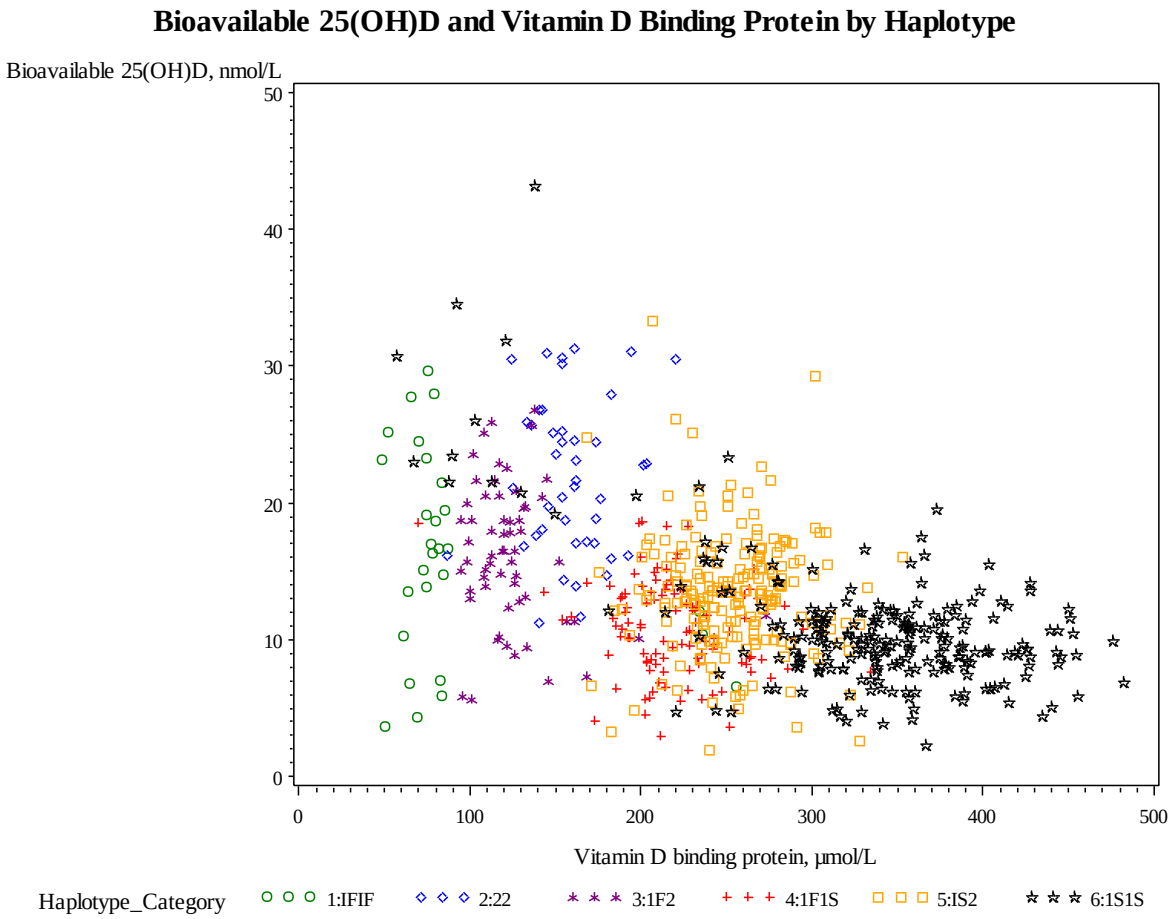


Scatter plot of Total 25(OH)D and Bioavailable 25(OH)D by Vitamin D Binding Protein Quartile



Quartile 1 = Lowest Concentration of Vitamin D Binding Protein

Scatter plot of Bioavailable 25(OH)D and Vitamin D Binding Protein by Haplotype



Bioavailable 25(OH)D and any falls confounding analysis

Base		Beta	RR	Change %
BioAQuartile	**1**	0.0905	1.0947215	
BioAQuartile	**2**	-0.0568	0.944783	
BioAQuartile	**3**	-0.4306	0.6501189	
Arthritis				
BioAQuartile	**1**	0.0741	1.0769145	0.016266
BioAQuartile	**2**	-0.0542	0.9472426	-0.0026
BioAQuartile	**3**	-0.4404	0.6437789	0.009752
Dizzy		0.1271	1.1355306	-0.03728
BioAQuartile	**1**	-0.0446	0.95638	-0.01227
BioAQuartile	**2**	-0.4162	0.6595483	-0.0145
BioAQuartile	**3**			
PASE		0.051	1.0523229	0.03873
BioAQuartile	**1**	-0.0629	0.9390374	0.006081
BioAQuartile	**2**	-0.4278	0.6519418	-0.0028
BioAQuartile	**3**			
Stroke		0.0872	1.0911149	0.003295
BioAQuartile	**1**	-0.0504	0.950849	-0.00642
BioAQuartile	**2**	-0.429	0.6511599	-0.0016
BioAQuartile	**3**			
Education		0.0726	1.0753003	0.017741
BioAQuartile	**1**	-0.0564	0.945161	-0.0004
BioAQuartile	**2**	-0.4509	0.6370545	0.020095
BioAQuartile	**3**			
Smoke				
BioAQuartile	**1**	0.0914	1.0957072	-0.0009
BioAQuartile	**2**	-0.0572	0.9444052	0.0004
BioAQuartile	**3**	-0.4297	0.6507043	-0.0009
Alcohol				
BioAQuartile	**1**	0.085	1.0887171	0.005485
BioAQuartile	**2**	-0.0512	0.9500886	-0.00562
BioAQuartile	**3**	-0.4345	0.6475884	0.003892
Total PTH				
BioAQuartile	**1**	0.1144	1.1212005	-0.02419
BioAQuartile	**2**	-0.0762	0.9266309	0.019213

BioAQuartile	**3**	-0.4262	0.6529857	-0.00441
BioT				
BioAQuartile	**1**	0.0836	1.0871939	0.006876
BioAQuartile	**2**	-0.0963	0.9081915	0.03873
BioAQuartile	**3**	-0.4057	0.6665101	-0.02521
Total T				
BioAQuartile	**1**	0.0921	1.0964745	-0.0016
BioAQuartile	**2**	-0.0431	0.9578156	-0.01379
BioAQuartile	**3**	-0.4124	0.6620594	-0.01837
Free T				
BioAQuartile	**1**	0.0872	1.0911149	0.003295
BioAQuartile	**2**	-0.0937	0.9105559	0.036227
BioAQuartile	**3**	-0.4042	0.6675106	-0.02675
Hypertensive Med				
BioAQuartile	**1**	0.0887	1.0927528	0.001798
BioAQuartile	**2**	-0.0598	0.9419529	0.002996
BioAQuartile	**3**	-0.4322	0.6490795	0.001599
Hypotensive Med				
BioAQuartile	**1**	0.0816	1.0850217	0.008861
BioAQuartile	**2**	-0.0416	0.9592534	-0.01532
BioAQuartile	**3**	-0.4312	0.649729	0.0006
Glucose				
BioAQuartile	**1**	0.0904	1.094612	1E-04
BioAQuartile	**2**	-0.0478	0.9533244	-0.00904
BioAQuartile	**3**	-0.4192	0.6575727	-0.01147
Cancer				
BioAQuartile	**1**	0.0913	1.0955976	-0.0008
BioAQuartile	**2**	-0.0591	0.9426125	0.002297
BioAQuartile	**3**	-0.4205	0.6567184	-0.01015
Angina				
BioAQuartile	**1**	0.0909	1.0951595	-0.0004
BioAQuartile	**2**	-0.046	0.955042	-0.01086
BioAQuartile	**3**	-0.4249	0.6538352	-0.00572
Parkinsons				
BioAQuartile	**1**	0.0957	1.1004289	-0.00521
BioAQuartile	**2**	-0.0654	0.9366927	0.008563
BioAQuartile	**3**	-0.4345	0.6475884	0.003892

SelfHealth				
BioAQuartile	**1**	0.0819	1.0853473	0.008563
BioAQuartile	**2**	-0.0574	0.9442163	0.0006
BioAQuartile	**3**	-0.4208	0.6565214	-0.00985
Outdoor				
BioAQuartile	**1**	0.0295	1.0299394	0.059177
BioAQuartile	**2**	-0.0767	0.9261677	0.019703
BioAQuartile	**3**	-0.4598	0.6314099	0.028778
MHFALL				
BioAQuartile	1	0.0665	1.068760964	0.023714
BioAQuartile	2	-0.0864	0.917227267	0.029166
BioAQuartile	3	-0.4272	0.652333073	-0.00341
CNS Meds				
BioAQuartile	1	0.0791	1.082312548	0.011335
BioAQuartile	2	-0.0648	0.937254896	0.007968
BioAQuartile	3	-0.4176	0.658625626	-0.01308
VitD Supp				
BioAQuartile	1	0.093	1.097461735	-0.0025
BioAQuartile	2	-0.0599	0.941858715	0.003095
BioAQuartile	3	-0.4375	0.645648526	0.006876

Bioavailable 25(OH)D and 2+ falls confounding analysis

Base		Beta	RR	Change %
BioAQuartile	**1**	0.1938	1.2138535	
BioAQuartile	**2**	-0.0235	0.976774	
BioAQuartile	**3**	-0.2096	0.8109085	
Arthritis				
BioAQuartile	**1**	0.1731	1.188985	0.020487
BioAQuartile	**2**	-0.0249	0.9754074	0.001399
BioAQuartile	**3**	-0.2245	0.7989156	0.01479
Dizzy				
BioAQuartile	**1**	0.2674	1.306563	-0.07638
BioAQuartile	**2**	-0.0053	0.994714	-0.01837
BioAQuartile	**3**	-0.1851	0.8310212	-0.0248
PASE				
BioAQuartile	**1**	0.1644	1.1786857	0.028972
BioAQuartile	**2**	-0.0268	0.9735559	0.003295
BioAQuartile	**3**	-0.2081	0.8121258	-0.0015
Stroke				
BioAQuartile	**1**	0.1905	1.2098544	0.003295
BioAQuartile	**2**	-0.0188	0.9813756	-0.00471
BioAQuartile	**3**	-0.2096	0.8109085	0
Education				
BioAQuartile	**1**	0.184	1.2020158	0.009752
BioAQuartile	**2**	-0.0259	0.9744325	0.002397
BioAQuartile	**3**	-0.2183	0.8038842	0.008662
Smoke				
BioAQuartile	**1**	0.2009	1.2225025	-0.00713
BioAQuartile	**2**	-0.0312	0.9692817	0.00767
BioAQuartile	**3**	-0.2044	0.8151363	-0.00521
Alcohol				
BioAQuartile	**1**	0.1932	1.2131254	0.0006
BioAQuartile	**2**	-0.0228	0.977458	-0.0007
BioAQuartile	**3**	-0.2099	0.8106653	0.0003
Total PTH				
BioAQuartile	**1**	0.2328	1.262129	-0.03977
BioAQuartile	**2**	-0.0245	0.9757977	0.001
BioAQuartile	**3**	-0.2069	0.813101	-0.0027

BioT				
BioAQuartile	**1**	0.187	1.2056273	0.006777
BioAQuartile	**2**	-0.0418	0.9590616	0.018134
BioAQuartile	**3**	-0.1754	0.8391213	-0.03479
Total T				
BioAQuartile	**1**	0.1694	1.1845939	0.024105
BioAQuartile	**2**	-0.0282	0.9721939	0.004689
BioAQuartile	**3**	-0.214	0.8073484	0.00439
Hypertensive Med				
BioAQuartile	**1**	0.1935	1.2134894	0.0003
BioAQuartile	**2**	-0.0241	0.9761881	0.0006
BioAQuartile	**3**	-0.2098	0.8107464	0.0002
Hypotensive Med				
BioAQuartile	**1**	0.1975	1.2183531	-0.00371
BioAQuartile	**2**	-0.0298	0.9706396	0.00628
BioAQuartile	**3**	-0.174	0.8402969	-0.03624
Glucose				
BioAQuartile	**1**	0.1931	1.2130041	0.0007
BioAQuartile	**2**	0.0071	1.0071253	-0.03107
BioAQuartile	**3**	-0.1741	0.8402129	-0.03614
Cancer				
BioAQuartile	**1**	0.1998	1.2211585	-0.00602
BioAQuartile	**2**	-0.0217	0.9785338	-0.0018
BioAQuartile	**3**	-0.1773	0.8375285	-0.03283
Angina				
BioAQuartile	**1**	0.1942	1.2143391	-0.0004
BioAQuartile	**2**	-0.0104	0.9896539	-0.01319
BioAQuartile	**3**	-0.2046	0.8149732	-0.00501
Parkinsons				
BioAQuartile	**1**	0.1938	1.2138535	1.1E-15
BioAQuartile	**2**	-0.0234	0.9768717	-0.0001
BioAQuartile	**3**	-0.2095	0.8109896	-0.0001
SelfHealth				
BioAQuartile	**1**	0.1396	1.1498138	0.052757
BioAQuartile	**2**	-0.0096	0.9904459	-0.014
BioAQuartile	**3**	-0.2141	0.8072677	0.00449

Outdoor				
BioAQuartile	**1**	0.1688	1.1838833	0.02469
BioAQuartile	**2**	-0.027	0.9733612	0.003494
BioAQuartile	**3**	-0.2192	0.8031611	0.009554
MHFALL				
BioAQuartile	1	0.147	1.158354	0.045722
BioAQuartile	2	-0.0768	0.9260751	0.051904
BioAQuartile	3	-0.2168	0.805091	0.007174
CNS Meds				
BioAQuartile	1	0.1618	1.1756251	0.031493
BioAQuartile	2	-0.0244	0.9758953	0.0009
BioAQuartile	3	-0.1937	0.823905	-0.01603
VitD Supp				
BioAQuartile	1	0.1871	1.2057479	0.006678
BioAQuartile	2	-0.0415	0.9593493	0.017839
BioAQuartile	3	-0.2192	0.8031611	0.009554

Table 8: Haplotype and DBP Analysis

Any Falls in first year by DBP Quartile

DBP Quartile	Range (µmol/L)	Any Falls, n (%)
1	49.04 - 198.92	47 (27.81%)
2	199.46 - 250.60	51 (30.54%)
3	250.80 - 311.00	41 (24.26%)
4	311.60 - 482.80	56 (33.14%)

Chi-Square(3)=0.31

Table 9: Any Falls in first year by Haplotype

Haplotype	N	DBP Mean, µmol/L, (SD)	Any Falls, n (%)
1F1F	28	91.35 (±54.31)	8 (28.57%)
1F1S	109	219.26 (±33.93)	28 (25.69%)
1F2	60	124.35 (±27.56)	17 (28.33%)
1S1S	231	333.53 (±75.47)	75 (32.47%)
1S2	203	254.82 (±32.81)	58 (28.57%)
22	43	158.76 (±24.12)	9 (20.93%)

Chi-Square(5)=0.65

Table 10: Adjustment of Final Model for DBP and Haplotype

Final Model	RR	95% CI		% Change
Q1	**1.09**	0.80	1.50	ref
Q2	**0.94**	0.68	1.30	ref
Q3	**0.65**	0.45	0.95	ref
Final Model adding DBP				
Q1	**1.03**	0.71	1.50	5%
Q2	**0.90**	0.62	1.30	5%
Q3	**0.63**	0.43	0.93	3%
Final Model adding Haplotype				
Q1	**1.01**	0.69	1.46	8%
Q2	**0.87**	0.60	1.25	8%
Q3	**0.61**	0.41	0.90	7%
Final Model adding DBP and Haplotype				
Q1	**1.04**	0.69	1.55	5%
Q2	**0.89**	0.61	1.32	5%
Q3	**0.62**	0.41	0.92	5%

*Adjusted for race, age, BMI, season of blood draw and latitude

Table 11: Models of DBP and Haplotype with any falls

Using 1S2 as referent - Most common haplotype in quartile 3

Independent Variable	RR	95% CI		p-value
Haplotype: 1F1F	**0.96**	0.47	1.96	0.92
Haplotype: 1F1S	**0.84**	0.57	1.23	0.36
Haplotype: 1F2	**0.91**	0.53	1.56	0.72
Haplotype: 1S1S	**1.16**	0.84	1.62	0.37
Haplotype: 22	**0.73**	0.38	1.40	0.34
Haplotype: 1S2	**Ref**	Ref	Ref	Ref
DBP	**1.00**	1.00	1.00	0.67

*Adjusted for race, age, BMI, season of blood draw and latitude

Using 1S1S as referent - Most common haplotype in entire cohort

Independent Variable	RR	95% CI		p-value
Haplotype: 1F1F	**0.83**	0.37	1.83	0.64
Haplotype: 1F1S	**0.72**	0.46	1.11	0.14
Haplotype: 1F2	**0.78**	0.41	1.47	0.44
Haplotype: 1S1S	**Ref**	Ref	Ref	Ref
Haplotype: 22	**0.63**	0.31	1.27	0.19
Haplotype: 1S2	**0.86**	0.62	1.19	0.37
DBP	**1.00**	1.00	1.00	0.67

*Adjusted for race, age, BMI, season of blood draw and latitude