Vitamin D time profile based on the contribution of non genetic and genetic factors in HIV-infected individuals of European ancestry

Running title: Vitamin D in HIV-infected individuals

Monia Guidi[1,2], Giuseppe Foletti[3], Paul McLaren[3], Matthias Cavassini[4], Andri Rauch[5], Philip E. Tarr[6], Olivier Lamy[7], Alice Panchaud[1,2], Amalio Telenti[3], Chantal Csajka[1,2]*, Margalida Rotger[3]* and the Swiss HIV Cohort Study

[1]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva; [2]Division of Clinical Pharmacology and Toxicology, University Hospital and University of Lausanne, Lausanne; [3]Institute of Microbiology, University Hospital and University of Lausanne, Lausanne; [4]Division of Infectious Diseases, University Hospital and University of Lausanne, Lausanne; [5]Division of Infectious Diseases, University Hospital Bern, Bern; [6]Infectious Diseases Service, Kantonsspital Basell and University of Basel, Basel; [7]Division of Internal Medicine University Hospital and University of Lausanne, Lausanne, Switzerland.

***Corresponding authors and requests for reprints, equal contribution to the work**

Prof Chantal Csajka

Division of Clinical Pharmacology

University Hospital Center

1011 Lausanne CHUV

Tel: + 41 21 314 42 63

Fax: + 41 21 314 42 66

chantal.csajka@chuv.ch

Dr. Margalida Rotger

Institute of Microbiology

University Hospital Center

1011 Lausanne CHUV

Tel: + 41 21 314 4102

Fax: + 41 21 314 4095

Margalida.rotger@chuv.ch

Word count: 3794

Abstract

Background: Vitamin D deficiency is prevalent in HIV-infected individuals and vitamin D supplementation is proposed according to standard care. This study aimed at characterizing the kinetics of 25(OH)D in a cohort of HIV-infected individuals of European ancestry to better define the influence of genetic and non-genetic factors on 25(OH)D levels. These data were used for the optimization of vitamin D supplementation in order to reach therapeutic targets.

Methods: 1,397 25(OH)D plasma levels and relevant clinical information were collected in 664 participants during medical routine follow up visits. They were genotyped for 7 SNPs in 4 genes known to be associated with 25(OH)D levels. 25(OH)D concentrations were analyzed using a population pharmacokinetic approach. The percentage of individuals with 25(OH)D concentrations within the recommended range of 20-40ng/ml during 12 months of follow up and several dosage regimens were evaluated by simulation.

Results: A one-compartment model with linear absorption and elimination was used to describe 25(OH)D pharmacokinetics, while integrating endogenous baseline plasma concentrations. Covariate analyses confirmed the effect of seasonality, body mass index, smoking habits, the analytical method, darunavir and the genetic variant in *GC* (rs2282679) on 25(OH)D concentrations. 11% of the interindividual variability in 25(OH)D levels was explained by seasonality and other non-genetic covariates and 1% by genetics. The optimal supplementation for severe vitamin D deficient patients was 300000 IU 2/year.

Conclusions: This analysis allowed identifying factors associated with 25(OH)D plasma levels in HIV-infected individuals. Improvement of dosage regimen and timing of vitamin D supplementation is proposed based on those results.

Key words: vitamin D, population pharmacokinetics, supplementation, HIV, genetic factors.

Introduction

Vitamin D deficiency is highly prevalent in HIV-infected individuals [1-4]. There are multiple factors that influence vitamin D physiology, most importantly exposure to sunlight, and thus, seasonality. A study performed in the Swiss HIV Cohort Study (SHCS) estimated that 42–52% of participants were deficient in vitamin D in spring and 14–18% in fall [5]. Other relevant factors in the general population are black skin, age, obesity, the presence of diseases and drugs influencing vitamin D metabolism and smoking habits [6].

Vitamin D plays a central role in bone metabolism by binding to the vitamin D receptor that regulates transcription of many genes [7]. The primary source of vitamin D is exposure to sunlight, with a less important role of food intake. It is synthesized in the skin and metabolized in the liver to 25-hydroxyvitamin D (25(OH)D) and hydroxylated in the kidneys to its active form 1,25-dihydroxyvitamin D (1,25(OH)D) [8]. Vitamin D status is best assessed by 25(OH)D concentration ([25(OH)D]) measurements [6, 9-11]. There is no consensus regarding optimal vitamin D status although most experts define vitamin D deficiency if [25(OH)D] are<20 ng/ml (<50 nmol/l). Plasma levels of about 30-40 ng/ml (75-100 nmol/l) have been associated with a decrease in mortality [6, 11-13]. An increase in mortality risk has been suggested at concentrations higher than 45 ng/ml (112.5 nmol/l) [14].

Three genome wide association studies (GWAS) have been performed in the general population of European ancestry, which consistently showed that single nucleotide polymorphisms (SNPs) in four loci (*GC, CYP2R1, DHCR7/NADSYN1* and *CYP24A1*) influenced vitamin D physiology. [15-17]. All four loci have biological plausibility: *GC* encodes the vitamin D binding protein (DBP) [18]. *CYP2R1* encodes a hepatic microsomal

enzyme involved in the 25-hydroxylation of vitamin D in the liver [19]. *DHCR7* encodes the enzyme 7-dehydrocholesterol (7-DHC) reductase, which converts 7-DHC to cholesterol, thereby removing the precursor of 25(OH)D [20]. *CYP24A1* encodes a 24-hydroxylase, which initiates degradation of both 25(OH)D and 1,25(OH)D [21].

Recently, Foissac et al. evaluated the impact of non-genetic factors on [25(OH)D] in HIV-infected individuals. Seasonality and skin color were identified as the only significant factors influencing 25(OH)D pharmacokinetics. Their study proposes a dosage regimen of 100000 IU of vitamin D monthly for optimal vitamin D status [22]. In the present study, we aim at characterizing the kinetics of [25(OH)D] in a cohort of HIV-infected individuals and to better define the influence of various genetic in addition to non-genetic factors on these levels. We then explored various vitamin D dosage regimens to propose adequate supplementation of vitamin D.

Material and Methods

Study participants.

Inclusion criteria were HIV-infected individuals of European ancestry, followed at 3 Swiss HIV Cohort Study (SHCS) centers that perform routine 25(OH)D testing during follow-up visits, who gave written informed consent for genetic testing and had at least one 25(OH)D plasma value between February 2009 - December 2012. The demographic, clinical and lifestyle characteristics, as well as the different vitamin D supplementation regimens, antiretroviral therapy (ART) and co-administered drugs were extracted from the SHCS database. The study was approved by the ethics committees of all participating centers.

Measurement of vitamin D plasma levels.

[25(OH)D] were quantified by either a liquid chromatography tandem-mass spectrometry (LC-MS/MS) (centers of Lausanne and Basel) or an automated chemiluminescent immunoassay (LIAISON® DiaSorin) (center of Bern) [23, 24].

Genotyping.

We selected 7 SNPs in 4 genes significantly associated with vitamin D plasma levels in three GWAS: rs12785878 and rs3829251 in *NADSYN1/DHCR7*; rs12794714 and rs10741657 in *CYP2R1*; rs2282679 and rs7041 in *GC*; and rs6013897 in *CYP24A1* [15-17]. Other SNPs identified in GWASs were not included because they were in high linkage disequilibrium (r2>0.8) with those selected. Genotyping was performed by TaqMan allelic discrimination using an Assay-on-demand® from Applied Biosystems. Genotyping results were verified by PCR and direct sequencing for two individuals per genotype. The Assay-on-demand® as well as the primers and PCR conditions are shown in **supplemental table 1**.

Population pharmacokinetic analysis.

The non-linear mixed effect modeling program (NONMEM®, version 7.2) [25] with the PSN-toolkit (version 3.5.3) [26, 27] was used to describe concentration-time profile.

Structural and statistical model.

[25(OH)D] collected at baseline and after vitamin D supplementation were employed for model development. A one-compartment model with first-order absorption and elimination was used the describe [25(OH)D], while incorporating endogenous production by estimating a baseline concentration (**Figure 1**), as follows:

$$\frac{d[25(OH)D]_{vitD}}{dt} = K_{12} * [vitD] - K_e * [25(OH)D]_{vitD} \qquad \text{Eq 1}$$

$$[25(OH)D] = C_{base} + [25(OH)D]_{vitD} \qquad \text{Eq 2}$$

where [VitD] represents the dose and $[25(OH)D]_{vitD}$ the resulting concentration, C_{base} the baseline endogenous 25(OH)D concentrations, [25(OH)D] the total concentrations in the central compartment and K_e and K_{12} the elimination and the biotransformation rate constants. The latter describes vitamin D absorption and metabolization into 25(OH)D. Estimated parameters were C_{base}, apparent volume of distribution of the central compartment (V) and apparent clearance ($CL = K_e \cdot V$). The mean biotransformation time was calculated using $1/K_{12}$ and the mean elimination half-life using $\ln(2)/K_e$.

Interindividual variability was described by exponential errors following a log-normal distribution. Proportional, additive and combined proportional-additive error models were compared to describe 25(OH)D residual variability. Distinct error models were tested to account for potential differences related to SHCS centers.

Covariate Analyses.

Demographic covariates (sex, age and body mass index (BMI)), seasonal variations, current smoking habits, alcohol consumption, HIV-infection status (duration of HIV infection, viral load (RNA) and CD4 cell count), chronic hepatitis C (HCV) and B (HBV), transaminases (alanine (ALT) and aspartase (AST) aminotransferases), ART medication and coadministration of rifampicin (the only drug reported in the SHCS database), genetic variants and the analytical method were tested on 25(OH)D kinetic parameters. The covariate analysis was performed using a stepwise insertion/deletion approach. All detected parameter/non-genetic covariate relationships were modeled using linear or non-linear functions as appropriate (categorical covariates coded as 0 and 1, continuous covariates centered on their median value). Log and square root transformations were respectively used for RNA and CD4 count; ALT and AST were coded into dichotomous variables employing a boundary condition of 1.5 times the upper limit of normal (ULN); alcohol consumption was dichotomized using different cutoffs: 20g/day (the recommended maximum alcohol intake in Switzerland), 30g/day or 40g/day. The seasonal variation of C_{base} was modeled by a cosine function of the day of the year (*DAY*) as illustrated by the following equation:

$$C_{base}=TVC_{base}*(1+AMP*\cos\left(2*\pi*\frac{DAY-DAY_{peak}}{365}\right)) \qquad \text{Eq 3}$$

where TVC_{base} represents the average concentration over a year and DAY_{peak} the day of the year at which the maximum covariate effect (*AMP*) occurs.

Individuals were categorized into genetic groups (common alleles (Ref), heterozygous (Het LOF) and homozygous (Hom LOF) loss of function). Parameters values were estimated for

each genotypic group (rich model) or for further regrouped (reduced model) sub-populations.

Parameter estimation and model selection.

The first-order conditional estimation (FOCE) method with INTERACTION was used for model fitting. The log-likelihood ratio test, based on the reduction of the objective function value (ΔOFV), was used to discriminate between hierarchical models. A change in the objective function was considered statistically significant if it exceeded 3.84 ($p<0.05$) and 6.63 ($p<0.01$) for 1 additional parameter in the model-building and backward-deletion steps, respectively (ΔOFV between any two models approximates a χ^2 distribution). Additional criteria for model selection were goodness-of-fit plots, precision of the parameters, and the reduction of interindividual variability.

Model evaluation and assessment.

The adequacy of the model was assessed by means of the bootstrap method (PsN), generating 2000 datasets by re-sampling from the original dataset. Mean parameters values with their 95% confidential interval ($CI_{95\%}$) were derived and compared with the final estimates. The predictive performance of the final model was evaluated by normalized prediction distribution errors (NPDEs) computation simulating each observation 3000 times [28].

Simulations.

Several vitamin D supplementation dosage regimens were simulated with NONMEM® for 1000 individuals based on the final model estimates with variability. [25(OH)D] were predicted and compared to the suggested range of 20-40 ng/ml (50-100 nmol/l) associated with optimal vitamin D status. Simulated dosage regimens were: 2000 IU daily, 4000 IU

daily, 300000 IU once/twice/three times per year (assuming equi-spaced time intervals), 800 IU daily with and without a 300000 IU loading dose and 100000 IU monthly as proposed by Foissac et al [22]. Simulations were performed considering either severe (10 ng/ml (25 nmol/l), range: 7.5-12.5 ng/ml)) or mild (20 ng/ml (50 nmol/l), range: 15-25 ng/ml) vitamin D deficiency. The range was derived from the estimated seasonal variation, which was the only covariate included in the model. Average and 95% prediction interval ($PI_{95\%}$) at minimal and maximal [25(OH)D] were calculated for each dosage regimen. Figures were generated with GraphPad Prism® (Version 6.00 http://www.graphpad.com/) and statistical analyses performed using R (Version 2.15.1, R Development Core Team, Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/).

Results

Study population.

A total of 664 participants provided 1,397 [25(OH)D], of which 783 were obtained before vitamin D supplementation (**Supplemental figure 1**). A median of two samples per individual (range: 1-5) was collected. Various vitamin D3 dosage regimens were utilized (300000 IU 1/year (n=493), 400-800 IU 1/day (n=33), 45000 IU 1/month (n=1) or in combined dosage regimens (n=15)). Data measurements were determined mostly by LC-MS/MS (84%). The median (range) 25(OH)D plasma level before vitamin D supplementation was 17.0 ng/ml (4.6-91.2 ng/ml). Vitamin D deficiency, i.e. [25(OH)D]<20 ng/ml (50 nmol/l), was present in 58% of the individuals, among whom 19% had levels<10 ng/ml (25 nmol/l). The median (range) HIV disease duration was of 11 years (0-29 years). All participants received ART: 91% contained NRTI and 12% received at least one PI with one NNRTI. Ritonavir (RTV) was administered as booster at a dose of 100 mg once or twice daily, except for one individual that received saquinavir with 400 mg RTV twice daily and one individual that received RTV 600 mg twice daily as a single PI regimen. Baseline characteristics of the study population are summarized in **Table 1**.

Genotyping.

Genotyping was completed for 658 participants. The minor allele frequencies (MAF) of the 7 SNPs were in Hardy-Weinberg equilibrium of p>0.05 except for rs10741657, which was excluded from the analysis. MAF was in accordance with results from HapMap for European populations: rs12785878 (MAF= 0.28), rs3829251 (MAF=0.16), rs12794714 (MAF=0.49), rs2282679 (MAF=0.27), rs7041 (MAF=0.44), rs6013897 (MAF=0.24). Sequencing confirmed the genotyping results. Since all genetic variants are well validated,

we created an unweighted genetic score by counting the number of risk alleles [29]. Because the literature frequently refers to *GC* haplotypes, two SNPs in *GC* (rs7041 and rs2282679, that tags the functional variant rs4588) were also tested (**Supplemental table 2**) [30].

Population pharmacokinetic analysis.

Structural model. A one-compartment model integrating endogenous concentrations adequately described 25(OH)D kinetics (**Figure 1**). Owing to the long time interval between dose intake and 25(OH)D measurements, [25(OH)D] were assumed to be at equilibrium and K_{12} was set equal to K_e. Assignment of an interindividual variability on C_{base} markedly improved the fit (ΔOFV =-579.1, p<0.001), but no additional variability on CL or V was significant (ΔOFV<0.04, p>0.83). A mixed error model was used to quantify residual variability, with different additive components according to the SHCS centers (ΔOFV=-14.2, p<0.001). Comparison of the goodness-of-fit plots stratified on the different vitamin D regimen suggests that the model works equally well independently of the dosage scheme. The basic parameters estimates with interindividual variability (CV%) were a CL of 2.60 L/day, a volume of 277 L and a C_{base} of 17 ng/ml (44%). The estimated biotransformation rate constant, reflecting both the vitamin D absorption and its metabolism into 25(OH)D, was 0.01 h^{-1}, resulting in a mean biotransformation time of approximately 100 days.

Covariate analyses. Univariate analyses were initially performed by testing non-genetic covariates on C_{base}. The effect of seasonality (Eq 3) resulted in a marked improvement of the model fit (ΔOFV=-196.3, p<0.001). Our model estimates a maximum change in [25(OH)D] of 48% between winter and summer, with peak concentration occurring in late August (**Figure 2**). A significant association was found between BMI and C_{base} that could be equally described using linear or allometric power functions (ΔOFV<-19.1, p<0.001).

The linear model was retained based on graphical exploration. A decrease of approximately 2% in C_{base} was predicted for one point increment of BMI with respect to the population median value (23.5 kg/m^2). Body weight and height were highly correlated with BMI and not further tested and female had 12% higher C_{base} compared to male subjects (ΔOFV=-6.1, p=0.014). A significant 9% reduction in C_{base} was observed in smokers compared to non-smokers (ΔOFV=-7.7, p=0.005). It also appeared that [25(OH)D] measured by immunoassay were 41% lower than by LC-MS/MS (ΔOFV=-81.8, p<0.001). An important decrease of 60% in C_{base} was observed under rifampicin administration, but it did not reach statistical significance, most probably due to power issues (n=2) (ΔOFV = -3.7, p = 0.054). Finally, administration of tenofovir (TDF), boosted PIs (including the administration of RTV alone) or darunavir (DRV/r) solely increased C_{base} by 9%, 10% and 16%, respectively (ΔOFV<-6.5, p<0.01), while no other drugs were significant (ΔOFV>-1.3, p>0.3). Multivariate analyses of the significant ART drugs discarded all medications except DRV/r. To disentangle the effect of DRV/r and boosted PIs, we tested the influence of the latter covariate in the sub-set of participants that did not receive DRV/r (n=560). The influence of boosted PIs did not remain statistically significant on C_{base} (ΔOFV = -2.6, p = 0.11), suggesting that the effect was entirely due to DRV/r administration. All the remaining factors were not associated with C_{base} (ΔOFV>-0.8, p>0.4). (**Supplemental table 3**).

Genetic analyses revealed that solely rs2282679 in *GC* significantly affected C_{base} (ΔOFV=-13.7, p=0.003). No impact of the other SNPs was observed (ΔOFV>-6.1, p>0.11). Hom LOF carriers of rs2282679 presented [25(OH)D] 25% lower than Ref and Het LOF individuals, and no difference could be observed between rs2282679 Ref and Het LOF individuals (ΔOFV=-0.6, p=0.44). The haplotype of the two *GC* SNPs, *i.e.* rs2282679 and rs7041, was also found to significantly influence C_{base} (ΔOFV=-17.3, p=0.004). However, multivariate analyses showed that the genetic variant rs2282679 accounted for the effect

of *GC* haplotype. The use of the genetic score did not improve the fit (ΔOFV=-10.9, p=0.21) (**Supplemental table 3**).

Multivariate analyses and backward deletion discarded the effect of gender and confirmed that of seasonality, BMI, smoking, the analytical method, DRV/r and the genetic variant rs2282679 on C_{base}. These covariates explained 12% of the interindividual variability in [25(OH)D]. Most of it was due to seasonality and the analytical method (ca. 20%) while other factors contributed less than 10%. The final model parameters' estimates together with their bootstrap estimations are given in **Table 2**.

Model evaluation and assessment.

The model was considered reliable since all the obtained parameter estimates lied within the bootstrap $CI_{95\%}$ and differed in less than 4% from the bootstrap median values. In addition, the good predictive performance of our model was confirmed by the normality of the distribution of the computed NPDEs (**Supplemental figure 2**).

Simulation.

The model-based simulations predicting [25(OH)D] for the various dosage regimen are presented in **Supplemental table 4** and **Supplemental Figure 3**. These simulations show that adequate yearly coverage (20-40 ng/ml or 50-100 nmol/l) can be achieved in 80% of the individuals with severe vitamin D deficiency by administration of 300000 IU 2/year or 2000 IU/day. A 800 IU/day dosage regimen is insufficient to bring [25(OH)D] within the optimal range without a loading dose of 300000 IU. Adequate concentrations were achieved in 90% of the individuals with mild deficiency after administration of a single vitamin D 300000 IU or 800 IU daily. dosage regimen would bring [25(OH)D] higher than 40 ng/ml for a prolonged time (more than six months) in at least 40% of the simulated individuals with mild or severe deficiency (**Figure 3**).

Discussion

This study characterized the concentration-time profile of 25(OH)D in a large cohort of HIV-infected individuals of European ancestry and quantified the influence of genetic variants on vitamin D physiology in addition to other factors. The results of study allowed building a strategy for vitamin D supplementation dependent of the level of deficiency, while taking into account seasonality and residual variability.

The estimated 25(OH)D elimination half-life of 62 days is in good agreement with previously reported data [9, 10, 22]. Average baseline concentrations were low and presented marked interindividual variability, revealing a substantial proportion of individuals with suboptimal 25(OH)D concentrations. As expected, exposure to sunlight had the strongest effect on 25(OH)D endogenous production, with highest values observed in August [31]. An important effect of BMI was observed as well. Vitamin D is a fat soluble prohormone that is stored in the adipose tissue, thus explaining the decrease in [25(OH)D] with increased BMI [3, 32, 33]. Smoking led to lower 25(OH)D levels, which could be potentially the consequence of increased hepatic metabolism [1, 34, 35]. The 4-hydroxylation of 25-hydroxyvitamin D has been shown to involve a CYP3A4-dependent pathway [36, 37]. We hypothesize that the influence of DRV/r on 25(OH)D could thus be mediated by a CYP3A4 inhibition. This effect needs however to be confirmed since it is in disagreement with reported data showing no association with DRV/r co-administration and no influence of ritonavir was observed [1]. Rifampicin, administered to two individuals, was associated with a marked decrease in 25(OH)D, probably through induction of CYP3A4 or other cytochromes [38, 39].

Our study did not find any influence of EFV on [25(OH)D]. The EFV-induced decrease in [25(OH)D] is inconsistent in the literature [1-4, 22]. Fox et al. reported increased [25(OH)D] after switching from an EFV to a DRV/r containing regimen [40] but it was not possible to discriminate the effect of the two drugs. Finally our study confirms that immunoassay techniques provide concentrations measurements lower than LC-MS/MS methods [23, 24] Both immunoassays and LC–MS/MS are widely used [41]; thus, clinicians must be aware of this inter-assay difference for the interpretation of 25(OH)D levels. The inclusion of the assay type in our model allows predicting [25(OH)D] in clinical laboratories using either technique. Concerning genetic influence, only the SNP rs2282679 in *GC* was found to influence [25(OH)D]. This SNP presented the strongest signal in the 3 GWAS analyses previously performed and tags the functional variant rs4588 [15-17, 42]. *GC* encodes DBP, the major plasma transporter of 25(OH)D and 1,25(OH)D [18]. Wang et al. showed that rs2282679 was associated with reduced DBP concentration, thus possibly allowing for increased elimination [17]. Experiments in DBP deficient mice showed that 25(OH)D is more rapidly metabolized and excreted from the body than in animals with no deficiency [43]. How alleles modulating DBP levels can affect [25(OH)D] remains to be elucidated. Other SNPs did not contribute to [25(OH)D] because of their effect size and low allele frequency. While considering all significant covariates, only a small fraction of the variability in [25(OH)D] could be explained. Among all, seasonality explained a major part of the variation in the concentration. However, individuals with a high BMI, smokers, underexposed to sun and carriers of the genetic variation in *GC* could be at particular risk of presenting very low [25(OH)D].

Mild or severe vitamin D deficiency is highly prevalent in HIV-infected individuals and vitamin D supplementation is largely prescribed by clinicians. However, there is a lack of

clear recommendations of optimal dosage regimen in this population. The recommended 800IU/day for adults [6, 11] or high intermittent dose administrations were mostly used in our population. Our simulations suggest that individuals with severe vitamin D deficiency would reach adequate [25(OH)D] if administered with 300000 IU twice a year, 2000 IU daily or 800 IU daily with a single 300000 IU loading dose. On the other hand, a single vitamin D supplementation of 300000 IU per year would be appropriate for HIV-infected individuals with mild deficiency, independently of the season (summer or winter). Higher dosage regimens and more frequent administration could lead to [25(OH)D] much higher than 40 ng/ml (100 nmol/l) over a prolonged period of time, which might put individuals at risk of higher mortality [14]. Single oral dose is a convenient strategy in HIV-infected individuals because it ensures adherence and does not increase daily pill burden.

The main limitations of the study are the lack of information about sunlight exposure, vitamin D dietary intake, no measure of DBP and no measure of parathyroid hormone that tightly regulates renal production of 1,25(OH)D. Since the GWAS data was only available for individuals of European ancestry, we limited our study to this population. The observational nature of the study generated sparse data that, associated with the prolonged time intervals between supplementation and measurement of 25(OH)D levels, limits the ability to distinguish between vitamin D absorption and conversion to 25(OH)D. However, the study was robust in terms of ascertainment, as the unbiased measurement of vitamin D levels was done for all participants during routine visits. In addition, optimal vitamin D status, consensually estimated to be 20-40 ng/ml (50-100 nmol/l) in the general population, remains a matter of debate and should be confirmed in the HIV population.

In conclusion, this study shows that [25(OH)D] are highly variable and affected in particular by seasonality and several non-genetic factors and by a SNP in *GC*. Adequate

supplementation should consider the level of vitamin D deficiency so as to adjust dosage regimens. Vitamin D supplements may be important in the context of long term care of HIV-infected individuals, with the goal of preventing disorders in bone metabolism. A role of vitamin D has also been debated in the context of immune function [44], which is of additional relevance in the setting of HIV infection.

Acknowledgement

This study has been financed within the framework of the Swiss HIV Cohort Study (SHCS#698) (http://www.shcs.ch/) supported by the Swiss National Science Foundation (SNF) (grant # 134277) and (grant #324730_141234). The data are gathered by the Five Swiss University Hospitals, two Cantonal Hospitals, 15 affiliated hospitals and 36 private physicians (listed in http://www.shcs.ch/31-health-care-providers). The authors thank Deolinda Alves and Raquel Martinez for technical help, Yannick Vallet and Yann Manet for assistance and the Vital-IT (http://www.vital-it.ch) Center for High-Performance Computing of the Swiss Institute of Bioinformatics for providing the computational resources for the population analyses. The members of the Swiss HIV Cohort Study are: Aubert V, Barth J, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of "Positive Council"), Hasse B, Hirsch HH, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F, Schmid P, Schultze D, Schüpbach J, Speck R, Staehelin C, Tarr P, Telenti A, Trkola A, Vernazza P, Weber R, Yerly S.

Conceived and designed the experiments: MG, AT, MR, CC. Organized the clinical cohort: MC, AR, PT, OL. Performed the experiments: GF. Analyzed the data: MG, AP, CC. Wrote the paper: MG, CC, MR. Reviewed the manuscript for important intellectual content: all authors. Approved of the final version: all authors.

Table 1: Characteristics of the study population.

Baseline Characteristic	Value (% or range)
Sex (men)	496 (75)
Median age (yrs)	48 (18-80)
Median body weight (kg)	71 (40-125)
Median height (cm)	173 (145-196)
Median BMI (kg/m^2)	23.5 (14.9-44.7)
Current Smoking (yes)	341 (51)
Alcohol Consumption (yes)	304 (46)
Average daily alcohol consumption (g/day)	10 (1-180)
Analytical method	
LC-MS/MS	551 (83)
Immunoassays	113 (17)
Liver Transaminases (>1.5*ULN)	
ALT	64 (10)
AST	37 (6)
Chronic Hepatitis (yes)	
HBV	431 (65)
HCV	174 (26)
Viral load (log10 copies/mL)	1.9 (1.3-6.2)
CD4 cell count (cells/µL)	585 (26-2325)
Protease Inhibitors (yes)[a]	
Atazanavir	9 (1)
Atazanavir/r	117 (18)
Darunavir/r	104 (16)
Fosamprenavir/r	4 (0.6)

Lopinavir/r	91 (14)
Ritonavir (non-booster)	1 (0.1)
Saquinavir	2 (0.3)
Saquinavir/r	28 (4)
Non-nucleoside Reverse Transcriptase Inhibitors [a]	
Efavirenz	267 (40)
Etravirine	168 (25)
Nevirapine	52 (8)
Nucleoside Reverse Transcriptase Inhibitors (yes)[a]	
Abacavir	130 (20)
Didanosine	5 (0.7)
Emtricitabine	400 (60)
Lamivudine	203 (31)
Stavudine	3 (0.5)
Tenofovir	379 (57)
Zidovudine	68 (10)
Entry and Integrase Inhibitors (yes)[a]	
Elvitegravir	2 (0.3)
Maroviroc	9 (1.4)
Enfuvirtide	2 (0.3)
Raltegravir	114 (17)
CYP3A4 inducers (yes)[a]	
Rifampicin	2 (0.3)
Genetic polymorphisms (Ref/Het LOF/Hom LOF) [b]	
NADSYN1/DHCR7 rs12785878	350/250/58 (53/38/9)
NADSYN1/DHCR7 rs3829251	468/173/17 (71/26/3)
CYP2R1 rs12794714	170/335/153 (26/51/23)

CYP2R1 rs10741657	274/325/59 (42/49/9)
GC rs2282679	353/260/45 (54/39/7)
GC rs7041	194/348/116 (29/53/18)
CYP24A1 rs6013897	386/230/42 (59/35/6)

ALT: alanine aminotransferase; AST: aspartase aminotransferase; BMI: body mass index; LC-MS/MS: liquid chromatography tandem-mass spectrometry; HBV: chronic hepatitis B; HCV: chronic hepatitis C; Ref: reference; LOF: loss of function; Het: heterozygous; Hom: homozygous.

[a] Calculated using participants that receive at least once the HIV drug

[b] Estimated in the genotyped subpopulation (n=658)

Table 2: Final population pharmacokinetic parameter estimates of 25(OH)D and their bootstrap evaluations.

Parameter [a]	Population mean		Bootstrap evaluation	
	Estimate	RSE [b] (%)	Estimate	95%CI
CL (L/d)	2.7	6	2.7	(2.4; 3.0)
V (L)	243	6	243	(203; 290)
K_{12} (d^{-1})	0.01	--	0.01	--
TVC_{base} (ng/ml)	20.6	3	20.6	(19.4; 21.8)
AMP [c]	0.25	6	0.25	(0.21; 0.28)
DAY_{peak} [c] (d)	226	2	226	(218; 235)
θ^{d}_{BMI}	-0.46	15	-0.47	(-0.67; -0.28)
$\theta^{e}_{DRV/r}$	0.18	31	0.18	(0.08; 0.27)
$\theta^{f}_{Immunoassay}$	-0.39	8	-0.39	(-0.47; -0.32)
$\theta^{g}_{Hom\ LOF\ rs2282679}$	-0.24	26	-0.24	(-0.34; -0.14)
$\theta^{h}_{Smokers}$	-0.13	22	-0.13	(-0.19; -0.07)
$IIV^{i}_{C_{base}}$ (CV%)	39	4	39	(35; 42)
σ^{j}_{prop} (CV%)	26	4	25	(22; 28)
$\sigma^{k}_{SHCS\ centers\ of\ Basel\ and\ Berne}$ (ng/ml)	3.6	12	3.6	(2.6; 4.8)
$\sigma^{k}_{SHCS\ center\ of\ Lausanne}$ (ng/ml)	2.0	28	2.0	(1.1; 3.0)

$$C_{base} = TVC_{base} * (1 + AMP * cos\left(2 * \pi * \frac{DAY - DAY_{peak}}{365}\right)) * \left(1 + \theta_{BMI}\frac{BMI - 23.5}{23.5}\right) * \left(1 + \theta_{\frac{DRV}{r}}\right) * \left(1 + \theta_{immunoassay}\right) * \left(1 + \theta_{Hom\ LOF\ rs2282679}\right) * \left(1 + \theta_{Smokers}\right)$$

[a] CL, mean apparent clearance; V, mean apparent volume of distribution; K_{12}, mean absorption rate constant set equal to K_e=CL/V; TVC_{base}, average 25(OH)D plasma level over a year

[b] Relative standard errors of the estimates (RSE) are defined as SE/estimate and are expressed as percentages. SE and estimate values were retrieved directly from the NONMEM® output files.

[c] AMP is the maximal seasonal variation occurring at DAY_{peak} (Eq 1).

[d] Decrease in $C_{baseline}$ under BMI doubling with respect to the population median BMI value.

[e] Increase in $C_{baseline}$ due to DRV/r co-administration.

[f] Decrease in $C_{baseline}$ if measurement performed by immunoassay.

[g] Decrease in $C_{baseline}$ in Hom LOF rs2282679 individuals.

[h] Decrease in $C_{baseline}$ in smokers

[i] Interindividual variability defined as CVs (%).

[j] Proportional component of the residual (intra-individual) variability defined as CVs (%).

[k] Residual (intra-individual) concentration

Figure 1: Compartmental model used to describe 25(OH)D plasma concentration-time profile. [25(OH)D]: total 25(OH)D concentration in the central compartment; CL: clearance; V: volume of distribution; K_{12}: absorption rate constant; K_e: elimination rate constant; C_{base}: endogenous production of 25(OH)D.

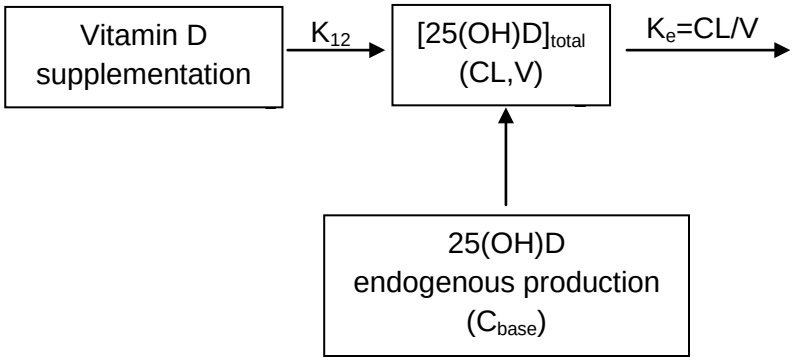

Figure 2: Observed baseline 25(OH)D concentrations (circles) versus time with average predicted concentrations (line)

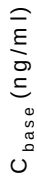

Figure 3: Simulated average 25(OH)D plasma levels (solid lines) with $PI_{95\%}$ (dashed lines) for various vitamin D supplementation dosage regimens in HIV-infected individuals with severe vitamin D deficiency. Panel A) 25(OH)D baseline concentrations ranging over a year from 7.5 to 12.5 ng/ml (average value 10 ng/ml (25 nmol/l). Panel B: 25(OH)D baseline concentrations ranging over a year from 15 to 25 ng/ml (average value 20 ng/ml (50 nmol/l). The recommended 25(OH)D concentrations range for optimal vitamin D status is shown (20-40 ng/ml or 50-100 nmol/l).

A

B

References

1. Allavena C, Delpierre C, Cuzin L*, et al.* High frequency of vitamin D deficiency in HIV-infected patients: effects of HIV-related factors and antiretroviral drugs. *J Antimicrob Chemother* 2012; **67:**2222-2230.
2. Brown TT & McComsey GA. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. *Antivir Ther* 2010; **15:**425-429.
3. Dao CN, Patel P, Overton ET*, et al.* Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis* 2011; **52:**396-405.
4. Welz T, Childs K, Ibrahim F*, et al.* Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. *AIDS* 2010; **24:**1923-1928.
5. Mueller NJ, Fux CA, Ledergerber B*, et al.* High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients. *AIDS* 2010; **24:**1127-1134.
6. Holick MF, Binkley NC, Bischoff-Ferrari HA*, et al.* Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; **96:**1911-1930.
7. Wang Y, Zhu J & DeLuca HF. Where is the vitamin D receptor? *Arch Biochem Biophys* 2012; **523:**123-133.
8. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; **80:**1689S-1696S.
9. Jones G. Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr* 2008; **88:**582S-586S.
10. Jones KS, Schoenmakers I, Bluck LJ, Ding S & Prentice A. Plasma appearance and disappearance of an oral dose of 25-hydroxyvitamin D2 in healthy adults. *Br J Nutr* 2012; **107:**1128-1137.
11. Brouwer-Brolsma EM, Bischoff-Ferrari HA, Bouillon R*, et al.* Vitamin D: do we get enough? A discussion between vitamin D experts in order to make a step towards the harmonisation of dietary reference intakes for vitamin D across Europe. *Osteoporos Int* 2013; **24:**1567-1577.
12. Rosen CJ, Abrams SA, Aloia JF*, et al.* IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab* 2012; **97:**1146-1152.
13. Zittermann A, Iodice S, Pilz S*, et al.* Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. *Am J Clin Nutr* 2012; **95:**91-100.
14. Michaelsson K, Baron JA, Snellman G*, et al.* Plasma vitamin D and mortality in older men: a community-based prospective cohort study. *Am J Clin Nutr* 2010; **92:**841-848.
15. Ahn J, Yu K, Stolzenberg-Solomon R*, et al.* Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet* 2010; **19:**2739-2745.
16. Lasky-Su J, Lange N, Brehm JM*, et al.* Genome-wide association analysis of circulating vitamin D levels in children with asthma. *Hum Genet* 2012; **131:**1495-1505.
17. Wang TJ, Zhang F, Richards JB*, et al.* Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 2010; **376:**180-188.
18. Speeckaert M, Huang G, Delanghe JR & Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin Chim Acta* 2006; **372:**33-42.
19. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ & Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci U S A* 2004; **101:**7711-7715.
20. Waterham HR & Wanders RJ. Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith-Lemli-Opitz syndrome. *Biochim Biophys Acta* 2000; **1529:**340-356.

21. Jones G, Prosser DE & Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys* 2012; **523:**9-18.
22. Foissac F, Treluyer JM, Souberbielle JC*, et al.* Vitamin D3 supplementation scheme in HIV-infected patients based upon pharmacokinetic modelling of 25-hydroxycholecalciferol. *Br J Clin Pharmacol* 2013; **75:**1312-1320.
23. Carrozza C, Persichilli S, Canu G*, et al.* Measurement of 25-hydroxyvitamin vitamin D by liquid chromatography tandem-mass spectrometry with comparison to automated immunoassays. *Clin Chem Lab Med* 2012; **50:**2033-2035.
24. Carter GD. Accuracy of 25-hydroxyvitamin D assays: confronting the issues. *Curr Drug Targets* 2011; **12:**19-28.
25. Beal S, Sheiner, L.B., Boeckmann, A., Bauer, R.J. *NONMEM User's Guides (1989-2009)*. 2009. Ellicott City, MD, USA.
26. Lindbom L, Pihlgren P & Jonsson EN. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Programs Biomed* 2005; **79:**241-257.
27. Lindbom L, Ribbing J & Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. *Comput Methods Programs Biomed* 2004; **75:**85-94.
28. Brendel K, Comets E, Laffont C, Laveille C & Mentre F. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharm Res* 2006; **23:**2036-2049.
29. Dudbridge F. Power and predictive accuracy of polygenic risk scores. *PLoS Genet* 2013; **9:**e1003348.
30. Braun A, Bichlmaier R & Cleve H. Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. *Hum Genet* 1992; **89:**401-406.
31. Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. *Prog Biophys Mol Biol* 2006; **92:**17-25.
32. Snijder MB, van Dam RM, Visser M*, et al.* Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *J Clin Endocrinol Metab* 2005; **90:**4119-4123.
33. Wortsman J, Matsuoka LY, Chen TC, Lu Z & Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; **72:**690-693.
34. Cutillas-Marco E, Fuertes-Prosper A, Grant WB & Morales-Suarez-Varela M. Vitamin D deficiency in South Europe: effect of smoking and aging. *Photodermatol Photoimmunol Photomed* 2012; **28:**159-161.
35. Hermann AP, Brot C, Gram J, Kolthoff N & Mosekilde L. Premenopausal smoking and bone density in 2015 perimenopausal women. *J Bone Miner Res* 2000; **15:**780-787.
36. Gupta RP, He YA, Patrick KS, Halpert JR & Bell NH. CYP3A4 is a vitamin D-24- and 25-hydroxylase: analysis of structure function by site-directed mutagenesis. *J Clin Endocrinol Metab* 2005; **90:**1210-1219.
37. Gupta RP, Hollis BW, Patel SB, Patrick KS & Bell NH. CYP3A4 is a human microsomal vitamin D 25-hydroxylase. *J Bone Miner Res* 2004; **19:**680-688.
38. Brodie MJ, Boobis AR, Dollery CT*, et al.* Rifampicin and vitamin D metabolism. *Clin Pharmacol Ther* 1980; **27:**810-814.
39. Wang Z, Lin YS, Zheng XE*, et al.* An inducible cytochrome P450 3A4-dependent vitamin D catabolic pathway. *Mol Pharmacol* 2012; **81:**498-509.
40. Fox J, Peters B, Prakash M*, et al.* Improvement in vitamin D deficiency following antiretroviral regime change: Results from the MONET trial. *AIDS Res Hum Retroviruses* 2011; **27:**29-34.
41. Su Z, Narla SN & Zhu Y. 25-Hydroxyvitamin D: Analysis and clinical application. *Clin Chim Acta* 2014; **433C:**200-205.

42. Johnson AD, Handsaker RE, Pulit SL*, et al.* SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; **24:**2938-2939.
43. Safadi FF, Thornton P, Magiera H*, et al.* Osteopathy and resistance to vitamin D toxicity in mice null for vitamin D binding protein. *The Journal of clinical investigation* 1999; **103:**239-251.
44. Hart PH, Gorman S & Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nat Rev Immunol* 2011; **11:**584-596.