Clinical aspects of vitamin D metabolism

PhD thesis

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Introduction

Vitamin D is a precursor of a hormone preserved through evolution for almost 1 billion year. The major physiological function of vitamin D in vertebrates is to maintain extracellular fluid concentrations of calcium and phosphorus within a normal range. The active vitamin D or D hormone controls directly as well as indirectly the expression of broad network of genes. Besides the active vitamin D has been shown to modulate a number of nongenomic, rapid responses through regulation of extranuclear signaling factors results in cellular responses that include increasing intracellular calcium, exocytosis/ATP secretion and UV protection. Active vitamin D is also a regulator of cellular proliferation and differentiation. Physiologic as well as potential therapeutic activities of the hormone are in cancer, in the regulation of immune system and autoimmune disease and in cardiovascular disease.

In VDR knock out mice the effect of Vitamin D cannot develop. They have growth retardation, severe rickets, osteomalacia, higher risk for different kinds of cancers, deficient immune system, cardiomyopathia, lipid abnormalities. In human besides the well known bone effects, the vitamin D deficiency has several extraosseal manifestations. It is associated with a higher risk of autoimmune diseases, of infectious diseases like tuberculosis or influenza, of cancer especially in the localization of colon and breast, of cardiomyopathy. Last, but not least it increases overall mortality.

There are still a lot of unanswered question about vitamin D deficiency. First of all there isn't an integrated internatinal normal reference range of 25OHD in the human serum. At the time of this writing, there is no consensus regarding dose range vitamin D supplementation during winter period when ultraviolet-B radiation is low therefore obtaining the adequate amount of sunlight is not possible. The recommended daily dose for adults is varies between 600 IU and 2000 IU. The Hungarian consensus of Vitamin D which is established by the agreement of experts on several different fields suggests that 75 nmol/l be the downer limit of the normal range and suggests that 2000 IU be the daily dose for adults during the winter months in Hungary for preventing vitamin D deficiency. It is unclear whether the duration of a vitamin D deficient period or its severity would pose a greater degree of risk for diseases. Similarly, there is uncertainty whether repeatedly declining levels of vitamin D levels in

temperate climate during the winter months would be associated with any health impairments. Epidemiological evidence suggests that vitamin D deficiency is reaching epidemic proportions with more than half of the world's population currently at risk. As the hungarian diet in general contains little vitamin D, and food is not fortified with it, the majority of vitamin D is formed in the skin by UVB radiation. Incident light barely contains any UV-B radiation from the 40th latitude to the poles from late autumn to spring, and is therefore unsuitable for vitamin D formation, so there is high risk for vitamin D deficiency in Hungary especially the end of winter. Therefore, serum vitamin D levels are gradually reduced during the winter months, reaching a nadir in the early spring period Although surveys have shown these seasonal fluctuations of vitamin D levels and the increased severity of winter deficit and even a mathematical model was created to predict seasonal fluctuation, all the above studies collected serum samples throughout different seasons of the year and no population-based estimate is available about the nadir of population-based vitamin D levels at the end of the winter period.

Due to its longer half-life, the total 25-hydroxilated-vitamin D (t-25OHD) levels are used to describe vitamin D status in clinical practice. However, vitamin D and its derivatives are mostly present in protein-bound form in the circulation, thus biologically disposable vitamin D levels are also dependent on the level of its binding proteins that are influenced by genetic and environmental factors. Consequently, bioavailable vitamin D (b-25OHD) and free vitamin D (f-25OHD) are expected to be more accurate markers of vitamin D deficiency. Still, there is a lack of evidence on representative studies that measured free or bioavailable vitamin D levels.

Vitamin D levels are known to be affected by several factors: sunshine, vitamin D supplementation, body mass index (BMI), age, place of residence, and use of indoor tanning facilities, however, it is unknown how these factors influence vitamin D levels by the very end of winter.

There are few studies testing the effectiveness of weekly and monthly supplementation lasting for winter period in which endogenous production of the vitamin is suppressed, however basic pharmacology principles suggest that the functional half-life of vitamin D_3 is a suitable for dosing weekly and monthly interval. Previous studies have shown that 1000 IU daily are considered a safety and minimally effective dose for treatment of vitamin D deficiency.

Objectives

My aims were to describe the frequency of vitamin D deficiency based on t-25OHD and b-25OHD, f-25OHD levels in Hungary at the end of winter, to investigate determinants of vitamin D levels including age, gender, place of residence, anthropometrics, occupation, vitamin D supplementation, and vitamin D binding protein (DBP), and to evaluate the suggested associations between vitamin D levels and pathological conditions (such as diabetes mellitus, hypertension, malignancy, autoimmune and cardiovascular diseases) suggested by the literature on a population-based representative sample of the Hungarian adults using samples collected during a one week period in early spring before significant amount of UV-B radiation was available in the country. I wished to investigate what is the relationship between serum calcium, phosphorus and parathormon levels and vitamin D values.

I wished to demonstrate efficacy and safety of three different vitamin D_3 dosing protocols with the same cumulative dose in vitamin D-deficient subjects for winter period time, which is three month long in Hungary. The groups were treated according to the following dose regimen: 1000 IU per day, 7000 IU per week and 30,000 IU per month.

I wished to compare the efficacy and safety of a higher dose by a weekly administration of 30 000 IU vitamin D_3 compared to the standard dose equivalent of 1000 IU/day in vitamin D deficient subjects.

Matherials and Methods

Study criteria and participants in the representative survey of total, bioactive and free vitamin D levels in the Hungarian adult population at the end of winter .

We aimed to sample a ten thousandth of the adult community dwelling population (n=831) in a representative way. No exclusion criteria was used.

We used a 2-stage sampling technique to draw our subjects. First, using the 2012 general practitioner (GP, n=6415) list of the National Public Health and Medical Officer Service, we invited a stratified random sample of 20 GP practices that corresponded to the distribution of the Hungarian adult population by residence (capital n=4, cities n=10, villages n=6) and geographical location (Central region [Budapest] n=4, Transdanubia n=8, Danube–Tisza Interfluve n=3, Transztisza n=5).

The second stage of the sampling involved a non-probability sampling of the people enlisted in the GP registry. As Hungary has a single insurer system with universal coverage of the entire population and people are either select a GP in proximity to their home or are automatically linked to the nearest GP practice, the sum of these GP lists cover the whole population of the country. At this stage, we followed a stratified quota sampling that corresponded to the age and sex (18-29 male 9%, female 9%; 30-59 male 27%, female 27%, 60+ male 11%, female 17%) structure of the Hungarian adult population. The quotas (to allow for missing data) were n=38 for the Budapest practices, n=45 for the city practices and n=42 for the village practices. The sampling was done in alphabetical order starting with the letter that was the first letter of the GP's family name. Patients who came to see the GP related to any actual medical problem were not included.

Medical history, anthropometrics (including weight and height), and vital signs were measured in the GP's office. All participants filled in a questionnaire regarding lifestyle habits associated with vitamin D metabolism, medical conditions and vitamin D supplementation. Planned date of sampling was adjusted to the expected hours of sunshine after the first days of March but preceding the date of two consecutive sunny days. This condition was fulfilled unusually late in 2013, during the first week of April.

Study criteria and participants in the randomized clinical trial to comparing efficacy of different dosing schedule of vitamin D_3.

This study is a controlled, randomized, four-armed, open label and multicenter clinical trial. Subjects were divided randomly into four groups. Each group was administered different doses of vitamin D_3 orally in the form of film-coated tablets. The doses included: 1000 IU/day for group A, 7000 IU/week for group B, 30,000 IU/month for group C, and a higher dose for group D which was 30,000 IU/week.

We selected community dwelling Hungarian adults with vitamin D deficiency (25OHD level < 50 nmol/l). Main exclusion criterias were hypercalcaemia, hypercalciuria, renal stone, metabolic bone diseases which is not osteoporosis, malabsorpcio, obesity, use of solarium, alcohol or drug abusion.

Five visits were made in a 90-day period. During the screening visit medical history was acquired by interview and a full physical examination was performed. Fasting blood samples were taken to determine study-specific lab parameters, including 25OHD, parathyroid hormone (PTH), serum calcium as well as routine chemistry. Urine samples were collected from fasting subjects at the second void of the morning. Data was collected data concerning adverse events, lifestyle and diet. These tests were repeated on the final visit. Calcium supplementation was provided by a registered product, if needed in case of low calcium intake of the subject.

Laboratory methods

The measurement of 25OHD and PTH was carried out by a direct, competitive chemiluminescence, immunoassay (CLIA, LIAISON analyzer DiaSorin, USA). The DBP levels were measured by immunoturbidimetry using polyclonal rabbit anti human Gc-globulin antibody (A0021Dako), Serum calcium, phosphate, alkaline phosphatase, and urinary calcium, phosphate were measured with a Beckman-Coulter automatic chemistry analyzer Au5800 (Beckman Coulter, Brea, USA). Urinary calcium excretion was assessed as a ratio of urinary calcium to creatinine concentrations.

The b-25OHD and f-25OHD values were calculated according to Camille E. Powe's mathematical model.

Statistical analysis

Sample weights were used to compensate for sampling errors related to nonresponse of GPs and missing samples. Descriptive data are given as means +/- standard deviations for continuous data and frequencies for categorical variables. Tests for normality were performed for all continuous variables and distributions were visually investigated using histograms of vitamin D levels and DBP.

We also investigated the association between the different measures of vitamin D and DBP using linear regression analysis with t-25OHD as the independent variable. Then, we investigated potential predictors of vitamin D related measures individually using linear regression models with vitamin D and DBP levels as independent variables. Those parameters that showed a univariate association ($p<0.1$) with vitamin D measures or DBP were subsequently made available for selection to a multiple linear regression model to describe independent determinants of these values using backward elimination.

All analysis was run on IBM SPSS Statistics version 20. A two-sided $p<0.05$ was considered statistically significant.

Power calculation was carried out to estimate the power of a one-way ANCOVA model with Dunnett *post hoc* test for pairwise comparisons (A vs. B; A vs. C). A sample size of 20 per treatment group was assumed and the power estimation was carried out by simulating and analyzing 5000 series of data.

Descriptive demographic statistics and statistics of safety parameters (plasma and urine Ca, PTH) on frequency and distribution of side effects were conducted by Kolmogorov-Szmirnov and Levene statistical tests. The efficacy of vitamin D_3 supplementation was determined by using Analysis of Covariance (ANCOVA) between the study groups.

Each dataset was analyzed using the clinical database software "Mythos" (Adware Research Kft., Hungary). After the CDC, - statistical analyses were accomplished using SPSS 19 (IBM Corporation, New York USA) and SAS 9.3 (SAS Institute Inc. Cary, USA) as specified by statistical reports.

Results

Results of the representative survey of total, bioactive and free vitamin D levels in the Hungarian adult population at the end of winter study

In our study, data from a representative cohort of the Hungarian population were processed. Due to missing questionnaire data and pre-analytical problems (such as broken tubes, or protocol deviations) the final analytical sample includes 664 people (29-61/GP practice).

The mean±SD of t-25OHD levels of the overall population was 41.27±20.6 nmol/l. In patients not receiving vitamin D supplementation, the corresponding values were 38.4±17.2 nmol/l. The figures did not follow a normal distribution. Ninety-four percent of the t-25OHD values of the total study population were below 75 nmol/l, 71% below 50 nmol/l and 30% did not reach 30 nmol/l. After excluding individuals receiving vitamin D supplementation (n=99), the incidence of the insufficiency substantially increased to 97%, 77%, and 34%, respectively.

The mean DBP was 311.1 ± 89.6 mg/l and it was approximately normally distributed. Although DBP and t-25OHD were significantly positively correlated (r=0,188 p<0,0001), DBP only explained 3% of the variance of vitamin D levels.

The mean b-25OHD value of the total population was 4.84 (± 4.33) nmol/l and the mean f-25OHD value was 11.83 (± 10,32) pmol/l. These two figures showed a high correlation with the t-25OHD (r=0,435 and r= 0,444, p <0.0001). The measured t-25OHD accounted for 58.5% and 62.2% of their values, respectively.

Neither t-25OHD nor b-25OHD or f-25OHD values showed an association with age but DBP levels decreased (r=0,165; p=0.001) with aging. Analysis of the effects of genders only justified differences in DBP: values were significantly (p= 0.001) higher in women.

The place of residence (Budapest, town, village) and the type of work (mental or physical labor work) did not affect vitamin D and DBP levels. We found also no correlation between the number of hours spent in the open air during the preceding winter or summer and vitamin D values. BMI was correlated with t-25OHD (r=0,115; p= 0.04) and DBP (r=0,283; p= 0.001), but showed no significant correlation with b-25OHD and f-25OHD values.

Although only a small percentage of the population went to sunbed sessions regularly (7%) or went to rest in a tropical country or in the mountains during winter (5%), places abundant in UVB radiation, both of these environmental factors had a significant impact on t-25OHD values ($p<0.0001$ and $p=0.009$). Beyond BMI, solarium usage, travel in tropics, vitamin D_3 supplementation ($p=0.004$) turned out to be independent predictors of t-25OHD values. Despite supplementation, 80% of vitamin D recipients had a value of less than 75 nmol/l, and 35% of less than 50 nmol/l.

Osteoporosis was diagnosed at the 7,8 % of the studied population. Their vitamin D levels were significantly higher ($p=0.02$) than in the general population, which was explained by vitamin D supplementation that is mandatory in Hungary along with the treatment of the disease. In our population, 48 % of osteoporotic patients were supplemented regularly with vitamin D. No correlation was found between the major complication of osteoporosis, i.e. former fractures and vitamin D levels tested.

Disease occurrence corresponded to the Hungarian disease incidences. Out of these disorders, only the incidence of cardiovascular diseases was found to be elevated with lower vitamin D values ($p=0.012$).

Results of the randomized clinical trial to comparing efficacy of different dosing schedule of vitamin D_3

A total of 140 subjects were screened and 89 were enrolled according to study criteria. There were 22-23 subjects in each group. There were not significant difference between the groups in vitamin D baseline values. 92% of the study subjects completed the study per protocol. Eight subjects dropped out. Compliance with operational framework of the study by the remaining subjects was excellent or good. We assessed the exact daily doses furthermore the rate of compliance by collecting unused medication and counting them at the time of each visit. The data sets produced by treatment administered to groups A, B, C and D were homogenous and exhibited be normal distribution. The duration of treatment was established at 84-90 days (for weekly or daily dosage groups) with a +/-8 days window of visit allowance. The treatment regimens consisting of a daily dose of 1000 IU (group A); versus weekly doses of 7000 IU (group B) and monthly doses of 30,000 IU (group C) demonstrated similar efficacy in normalization

of 25OHD levels in vitamin D-deficient subjects. Serum 25OHD concentrations increased by 30,6±3,5; 35,3±3,3 and 37,2±2,7 nmol/l in treatment groups of A, B and C, respectively. The measured daily equivalent vitamin D_3 intakes were 949±20 IU in group A, 1157±55 IU in group B and 1175±62 IU in group C, respectively. Thus, the corrected dose-response was almost the same in these three groups, 32,6±3,7; 31,6±2,8 and 32,3±2,3 nmol/l increase per 1000 IU/daily, respectively. The treatment of vitamin D-deficient subjects with different treatment regimens of 1000 IU/daily dose were judged to be equally effective in restoration of 25OHD values to above 50 nmol/l. Nevertheless, the mean levels failed to attains the 75 nmol/l threshold.

The statistical stratification by baseline 25OHD values, confirmed a higher increase in groups with lower baseline values. This observed effect is consistent with previously reported observations. The power of the statistical tests may have been limited, since a significant disparity between the size of groups was noted: 22,3 % enrolled with 25OHD < 25 nmol/l, and 77,7 % enrolled with levels between 25 nmol/l < 25OHD <50 nmol/l. The increase in 25OHD < 25 nmol/l group was Δ=44,08±16,5 nmol/l and for the higher baseline group, it was Δ=37,93±17,5 nmol/l.

The administration of higher dose (30 000 IU/week) was more effective compare to the 1000IU equivalent daily dose. Group D had significantly higher value by the end of the study compare to group A, B and C. Most of the subjects including the lower and the higher baseline subgroups in group D exceeded the limit of 75 nmol/l in 8 weeks.

Enrollment date-related variations were evaluated when the database had undergone a separation by screening dates. The two categories were designated Cohort I: March-April and Cohort II: May-June. The sample sizes were 25 vs. 36 subjects in these subgroups. There was no difference in serum 25OHD levels in these two cohorts at screening (33,63±9nmol/l, vs. 33,25±9,5 nmol/l). The potential for a periodic bias in vitamin D levels, if observed, should be treated as similar effect manifested for all subjects independently from treatment (since all enrolment subjects had deficiency as default by inclusion criteria). Taken together, the results confirmed that there is no bias detected between the two groups at any time point during the treatment period.

The overall mean of the two groups showed a difference with less than 1.0 nmol/l at any consequent visit dates.

Treatment-related changes in serum Ca and urinary Ca in each dose group and between the groups were evaluated. We did not find any difference in serum Ca in the groups and between the groups during the study. Since urinary calcium levels may have transient values during the day, and the determination of 24-hour-collected urine for was not feasible to perform for this study, instead the Calcium/Creatinine ratio has been determined at each time. Each elevated levels of urinary calcium have been reported as laboratory adverse event. The overall mean of serum PTH at screening was 49.4±16.4 pg/ml, which decreased to 44.7±17.3 pg/ml at the end of study, with no statistically significant difference among treatment groups.

There was no serious adverse event reported in the overall study. No dropouts occurred due to adverse events (AE), and no significant difference was found in reported AE and laboratory parameters among treatment groups. The most common AE reported were urinary calcium level increase , increase in urinary Ca/creatinine ratio (3 cases), increase in serum calcium level (2 cases). There was no significant difference in AE frequency among treatment groups.

Discussion

Representative survey of total, bioactive and free vitamin D levels in the Hungarian adult population at the end of winter study

We have found the most severe vitamin D deficiency due to environmental effects at the end of winter in a representative quota sample stratified for age, gender and place of residence. Our data showed lower values than previous studies conducted on a smaller number of healthy subjects in Hungary, Northern Europe, or a little further south of us, in Boston. Values of healthy adult subjects were similar or lower only in populations living far north of Hungary.

Low values can be explained by the time of sampling, the representative nature of the adult population, and the Hungarian diet. It is known that the half-life of the t-25OHD in the serum is 2-3 weeks and 2-3 month in human body. Therefore, it does not matter in which period of winter the samples were collected because vitamin D production in the skin is negligible in the entire winter period. A measurement taken 2-3 months before the end of winter can show a higher level of t-25OHD due to the gradual depletion of the previously accumulated t-25OHD amount. The difference can also be explained by local diet. While the normal diet of countries closer to the sea contains a greater amount of vitamin D, in Hungary the amount of vitamin D in food is significantly lower (80 IU/day), and dishes are not enriched with vitamin D.

Since our study was representative of age and gender, we were able to examine the population distribution of vitamin D values. Minimum value necessary for health is still debated. Institute of Medicine determined the lower limit of the normal range at 50 nmol/l. The American Endocrine Society, the International Osteoporosis Foundation and the Hungarian Consensus Meeting set this lower limit to 75 nmol/l. In most cases, 30 nmol/l is the lower limit for serious deficiency. We applied this value in our study.

The distributions of the t-25OHD, b-25OHD and f-25OHD curves did not follow a normal distribution at the end of the winter. The vast majority, 97% of the adult population not receiving vitamin D supplementation did not reach the 75 nmol/l, and 77% failed to reach the 50 nmol/l limit. More than one third of the population was in the seriously deficient range. These rates were much lower than the sample population's rates in the U.S. and in Ireland. The main

difference between sample rates can be due to the timing of sampling. While in the previous surveys, „winter" testing samples were obtained between November and March, we were timing the study procedures to the tail-end of the lowest UVB radiation period. Our results showed that in these latitudes, almost the whole adult population without vitamin D supplementation becomes vitamin D deficient and severe deficiency becomes shockingly frequent.

We measured DBP and albumin values at the same time, and calculated b-25OHD and f-25OHD levels. Vitamin D has no known effect on DBP values. Neither the DBP gene, nor genes of other proteins that influence the metabolism of DBP have a vitamin D-response element. However, DBP may significantly affect t-25OHD levels. In DPB deficient mice, t-25OHD values are extremely decreased without any clinical sign of vitamin D deficiency. The t-25OHD levels appear to be regulated partly by the DBP concentration. In a study by Powe at al, the DBP concentration seemed to be under tight genetic control and genetic alterations explained 9,9% of the variation in t-25OHD levels. In our study, DBP closely correlated with the value of t-25OHD, but it only explained 3% of the changes.

So far, few data were available about which environmental factors affect the severity of vitamin D deficiency in a long-term absence of sunlight. Contrary to former data, age or gender did not affect the level of t-25OHD in winter period in our study. The reason for this contradiction could be that by the end of winter, the effect of influential factors is significantly reduced and thus, do not distort the results in our case. According to our data, neither staying outdoors during the winter, nor sunbathing the previous summer, nor the place of residence, nor the type of work affected vitamin D values at the end of winter. As in previous studies, vitamin D levels of individuals who had traveled in the preceding three months to a place where UV-B radiation level was higher than in Hungary, proved to be higher. Our data confirm that people attending tanning salons regularly had significantly higher vitamin D levels, although modern tanning bed UV radiation was maximized in 0.3 W/m^2 3 years ago, and only a fraction of radiation is allowed to be UVB type.

Our data supported the known relationship between BMI and t-25OHD: a higher BMI is associated with lower t-25OHD levels. This relationship is explained by the fact that fat-soluble vitamin D is distributed in a larger volume of fat tissue. Lifestyle factors have also

been raised as possible explanation because overweight people tend to exercise less, spend fewer time in direct sunlight, and their clothes usually cover a bigger area of their body. In our study, however, these factors were negligible compared to the lack of UVB radiation. It is unknown at present that the mathematical relationship between t-25OHD and BMI carries a biological significance, and whether a lower t-25OHD level is associated with less free hormones. Since BMI was closely correlated with DBP, but not with b-25OHD and f-25OHD values, our results question the biological significance of lower t-25OHD associated with larger BMI. . In woman the f-25OHD value was lower and the DBP was higher at the same t-25OHD level. It may result more serious statement of the vitamin D deficiency.

Vitamin D supplementation is strongly correlated with vitamin D values. Although the Hungarian professional consensus recommends taking it during the winter months, only 15% of the total adult population took additional vitamin D at varying doses and frequency. Our study draws attention to the fact that in real life, even among those who are taking vitamin D supplementation, deficiency is very widespread.

Previous observations show association between vitamin D values and frequency of fractures, number of falls, and frequency of infectious, autoimmune, cardiovascular and malignant diseases. Our measured vitamin D levels did not correlate with the frequency of fractures, number of falls, and frequency of infectious, autoimmune and malignant diseases at the end of the winter. We did not find a relationship between diabetes mellitus and vitamin D levels either. Our negative results may be explained either by the lack of real relationship between different environmental factors and vitamin D deficiency or may indicate that not only the extent but the length of the deficit may also have an impact. At the same time, we could demonstrate a significant correlation between vitamin D deficiency and cardiovascular diseases, however, the questionnaire did not ask for specific details about this diagnosis group. Our observation is in agreement with earlier longitudinal data in which very low levels of t-25OHD were associated with a 1,2-2,5-fold increase in cardiovascular event risk. In a vitamin D intervention trial, this relationship was not verified, however, the cardiovascular events were not included in the primary endpoints of the study. Large randomized controlled trials are

required to clarify the suggested association between vitamin D status and cardiovascular diseases.

Serum calcium correlated with none of the 25OHD values. Serum phosphorus and parathormon values correlated with t and f- 25OHD, but after investigate them with ROC analisis, they did not have enough specifity nor enough sensitivity to use in laboratory testing to detect vitamin D deficiency or show the severity of the disease.

Our study has certain limitations. First, due to its cross-sectional and observational nature, we were unable to predict the future effects of extremely low t-25OHD level on the risk of diseases. Second, although GPs supervised the questionnaire survey of retrospective data about environmental factors and medical history, those were not validated. Third, we calculated and not directly measured b-25OHD and f-25OHD levels. Nevertheless, in other studies the calculated b-25OHD and f-25OHD well correlated with measured levels. The main advantages of the study include the representativeness of the sampling, the specific definition of sampling time and the calculated b-25OHD and f-25OHD values in this representative population.

The randomized clinical trial to comparing efficacy of different dosing chedule of vitamin D_3

Outcomes of our investigation demonstrated similar efficacy and safety profile of daily, weekly and monthly dosing of vitamin D_3 in a prospective, randomized clinical trial.

Ish-Shalom et al., reported similar results in elderly women who had undergone surgery to repair hip fracture. However, the present study differed in several respects. The population investigated here was younger and closer to the mean age of the Hungarian adult population. The present study utilized vitamin D tablets available for purchase by the public - as opposed to a specialized (noncommercial) vitamin D ethanolic solution. Additionally the study reported here was in excess of the 8 week period used by other investigators - and utilized a three month time period to match winter months in Hungary, during which sunlight exposure to most Hungarians is significantly reduced relative to summer months. Finally, a lower daily equivalent dose was utilized here, since 1000 IU is considered safe and minimally effective dose for treatment of vitamin D deficiency.

Our finding differs from report by Chel et al. in which a daily dose was more effective than a weekly dose, and a monthly dose was the least effective. In their randomized clinical trial elderly nursing home residents received 600 IU daily equivalent vitamin D_3 daily, weekly or monthly for 4 months. We suggest that their lower weekly and monthly administration efficacy was due to the lower compliance, because only random samples of the returned medication were counted in order to verify compliance. Besides this, thirty-nine percent of the nursing staffs reported the impression that fewer mistakes were made using daily instead of weekly or monthly administration. In contrast, in our study the compliance was determined at all visits. The compliance was excellent or good in every case. Based on our results the higher dose vitamin D_3 tablets can absorb properly as well.

Our study was the first where 1000 IU/day equivalent vitamin D_3 was given to a vitamin D deficient, healthy, community-dwelling adult population for 3 months in a prospective randomized clinical trial. Our dose-response rate was 32,5 nmol, slightly higher than it was expected based on the Endocrine Society's Clinical Guidelines, and almost the same as it was predicted by the Institute of Medicine estimation. Studies of similar daily dose have been published focusing mainly on osteoporotic and elderly patients. In earlier studies administering daily 1000 IU, dose-response rates varied within a wide range between 18-44 nmol/l. These differences may be explained by differences in baseline serum 25OHD levels and the different duration of vitamin D supplementation. Our study showed that 1000 IU daily equivalent dose for three months in vitamin D deficient population may elevate the mean 25OHD level above 50 nmol/l, but cannot reach 75nmol/l. With the higher dose of 30 000 IU/week the group exceeded the 75nmol/l limit within two months.

PTH response to vitamin D_3 replacement was not informative in the present study, since clinically significant PTH elevation was an exclusion criterion. Outcomes show that all PTH values were within the reference range and reduction of this parameter during the study was not significant, however, a marked trend toward a PTH decrease was nevertheless evident. The effect of vitamin D_3 replacement on serum 25OHD levels failed to exhibit seasonal fluctuations, presumably due to the use of sunscreen lotion during periods of normal-to-high sunlight exposure.

During the past decade, increasing evidence has mounted, that high annual doses of vitamin D may be harmful and due to long whole body half-life for this compound**,** daily dosing is generally considered unnecessary, therefore monthly or weekly dosing seems ideal. In our study, a weekly 7000 IU, monthly 30 000 IU and weekly 30 000 doses for 3 month in vitamin D deficient population was safe. Serious adverse event and drop out due to adverse event did not occur. We did not find any difference in adverse event frequency among 1000 IU daily equivalent dose and the higher weekly 30 000 IU dose treatment groups. This finding did not differ from earlier data. Recently published data reveal that higher dose vitamin D_3 supplementation, weekly 20,000 and 30,000 IU are safe for one year among women with low 25OHD level and high risk for breast cancer.

Our study has certain limitations. First, the number of participants was small compared to the number of study groups; however, the study has sufficient statistical power as it was calculated in advance. Second, due to the specific exclusion criteria, our safety data do not reflect all aspects of real life. Third potential limitation of the study is that it was not blinded. Although blinding was not necessary because all patients received the active agent, and because the outcome of the experiment, serum 25OHD, could not have been modified through a placebo effect. Fourth limitation was the relatively short study period. Longer study duration would have produced higher 25OHD levels, but this three-month equal to the length of winter in Hungary, when vitamin D_3 supplementation recommended for everyone in Hungary. Fifth limitation is that we did not investigated clinical outcomes, but those were beyond the scope of what we had intended to accomplish with this study.

The main advantages of our study include the prospective, controlled, randomized, multicenter clinical trial nature and the specific dosing schedules.

Conclusion

In summary, we have observed extremely low vitamin D levels on a representative sample during the period immediately prior to the emergence of spring sunlight. By the end of winter, almost the entire Hungarian population - without vitamin D supplementation - becomes vitamin D deficient. The degree of deficiency was not affected by age, gender, place of residence of the subjects or the number of hours spent outdoors, but was affected by tanning in a solarium, and travels sunny abroad. BMI only correlated with t-25OHD values, but did not affect b-25OHD and f-25OHD values. In woman, the f-25OHD value was lower and the DBP was higher at the same t-25OHD level. Among the diseases previously associated with low vitamin D levels, only cardiovascular diseases showed a significant correlation with the seasonally lowest vitamin D values. Serum calcium level did not correlate vitamin D levels. Serum level of, phosphorus and parathormon did not showed enough specifity nor enough sensitivity in laboratory testing of vitamin D deficiency.

Foremost we demonstrated equal efficacy and safety of the daily dose of 1000 IU, once weekly dose of 7000 IU and once monthly dose of 30,000 IU vitamin D_3 tablets treatment. The higher dose of 30 000 IU/week was more effective comparing to 1000 IU/day equivalens dose regimen, and the safety parameters of the higher dose (30 000 IU/week) were not different from the standard dose (1000 IU/day) in vitamin D deficient, healthy, community-dwelling adult population for 3 months in a prospective randomized clinical trial.

Publications *Summary of impact factors:9,339*

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