

Study on 25-Hydroxy-Vitamin D for the Assessment of Bone Mineral Density

MIHAELA CRACIUNESCU¹, DANA STOIAN^{1,*}, ADALBERT SCHILLER¹, MARIUS CRAINA¹, IZABELLA PETRE¹, ELENA BERNAD¹, FLORINA ANDRICA², FLORIN BORCAN², BOGDAN TIMAR¹

¹Victor Babes University of Medicine and Pharmacy Timisoara, Faculty of Medicine, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

²Victor Babes University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

Vitamin D is essential for the musculoskeletal health, its effect being independent of age, menopause or PTH level. This study aims to find the threshold of 25-OH vitamin D, suggestive for osteoporosis. We evaluated 341 postmenopausal women, aged 29 to 84 years and referred to the Center for Osteoporosis starting December 2014. All patients performed Dual X-Ray Absorptiometry (DXA) measurement. FRAX and 25-OH-D levels were determined. The mean 25-OH-D value in the investigated group was 23.69 ± 11.40 ng/mL (4.1-71.3 ng/mL). When we analyzed the DXA results, the hip and spine density turned out to be lower in the presence of vitamin D insufficiency. Using the ROC curve and Youden index, we observed that a 25-OH-D level lower than 21.80 ng/mL is highly predictive for lumbar and femoral neck bone mineral density. This evaluation is important in the assessment of bone health in postmenopausal women, decreased levels of vitamin D being associated with increased prevalence of bone demineralization.

Keywords: bone mineralization, DXA, vitamin D, vitamin D threshold

Vitamin D is essential for the development and maintenance of the skeletal health [1]. Many studies published in the last decade support the idea that vitamin D deficiency/insufficiency is associated with impaired quality of the bone [2-7]. On the other hand the bone protection is also dependent on calcium. Prevention of calcium removal from the skeletal buffer [8], reduction of the excessive bone remodeling [9], (having as an effect a reduction in bone fragility and fracture rates [10, 11] are essential bone protective mechanisms.

Aging is frequently accompanied by vitamin D deficiency/insufficiency due to reduction of solar exposure, decrease of intestinal absorption, and hepatic activation [12-15] decrease of renal activation (due to the aging kidney) [16], and vitamin D substrate deficiency due to nutritional errors [17].

A high prevalence of vitamin D deficiency/insufficiency was reported in people from Central European countries, but data from Romania are scarce [18-20]. There are also few data regarding the impact of vitamin D deficiency/insufficiency on bone mineralization in our area (46°N, 21°E). The reasons for the high prevalence of vitamin D deficiency are still unclear. Latitude, prevailing weather conditions, inadequate sun exposure, dietary and dress habits, access to healthcare, different food fortification policies and adherence to public health are the explanation recently found in a overview analysis [21].

The aim of this study was to assess the impact of vitamin D levels on bone mineralization in postmenopausal women, with or without osteoporosis.

Experimental part

The study was performed on 341 natural or surgical postmenopausal women, mean age 58.18 ± 9.01 years, referred for DXA evaluation, in the Center for Osteoporosis, between December 2014 and June 2015. The protocol was reviewed and approved by university ethical committee

(UMFT Ethical committee Agreement no. 26/2014) and the subjects were recruited through advertisement and personal invitation. Subjects were informed about the goal of this study, but they were not advised with regard to how performance was to be quantified. All the subjects gave their informed consent. Inclusion criteria were: naïve postmenopausal women, regardless the DXA results. Exclusion criteria were: long use of calcium supplementation (more than 2 months of at least 500 mg Calcium daily), any use of anti osteoporotic treatment (Bisphosphonates, Strontium Ranelat, Denosumab or Teriparatide) or hormonal replacement therapy, chronic use of medication with known bone impairment effects, metabolic bone disease, endocrine diseases, known malignancies, previous chemotherapy or radiotherapy, chronic kidney disease, renal phosphate leak, Paget's disease. DXA and lab investigation were performed on the same day.

Serum concentrations of 25-OH-D (D2 + D3) were measured for all patients at the same laboratory unit, the M&C Medical Laboratory, using a CLIA method, with an inter assay coefficient of variation of 5.5-9.2%. Blood samples were taken only in the morning, under fasting conditions. The minimum detection limit of the method is 4 ng/mL. The vitamin D sampling was made in maximum 1 week after the DXA evaluation. There is a general consensus supporting the idea that blood 25-OH-D levels below 10 ng/mL qualify as 'deficient', but beyond this there is currently no standard definition or agreement as to 'optimal' 25-OH-D levels [22, 23].

We used the threshold recommended by the current Central European Consensus [24], where Ukraine and Hungary, two states our country have direct border to, did participate. Unfortunately due to the lack of sufficient data, Romania was not part of the Consensus. Optimal 25-OH-D level for bone health is considered, at least by some researchers 30 ng/mL, value also used by us in this study [25, 26].

* email: stoian.dana@umft.ro; Tel: 0040.742.012.681

Serum values lower than 30 ng/mL were defined as vitamin D insufficiency [24], and values lower than 10 ng/mL were defined as vitamin D deficiency [24]. Levels >50 ng/mL were considered sufficient [27]. The same thresholds are recommended by the Endocrine Society [28].

Dual-energy X-ray absorptiometry measurements were performed in all cases, on the same device, DEXA T osteodensitometer, Osteosys Company (South Korea), a pencil beam antero-posterior device. The calibration of the DXA system was made daily with the European Spine Phantom. All measurements were performed and interpreted by the same technician, ISCD licensed since 2008.

Statistical analysis was performed using the NCSS version 9.0 for Apple (NCSS LLC, Utah, USA). Data are presented as mean, SD, SE, 90%LCL and 90% UCL. The distribution of all variables was tested with the Kolmogorov-Smirnov test. Unpaired T test was performed when comparing cases. Spearman's correlation was used the study of BMD in respect to 25-OH-D and age. Multiple regression models were used to evaluate the impact of different factors on bone mineral density. The ROC curve test was used to determine the vitamin D threshold. Written consent to use data in medical research was obtained from all participants.

The Ethics Committee of the Center for Osteoporosis

Results and discussions

Vitamin D: results in 341 postmenopausal women

The 25-OH-D values ranged from 4.1 to 55.0 ng/mL, with a mean annual serum 25-OH-D values were 23.69 ± 11.40 ng/mL. Vitamin D insufficiency was detected in 65.39% of the investigated group (223 cases) and deficiency in 9.38% (32 cases). Only 86 patients (25.21%) had normal 25-OH-D values.

As expected, there was an increase of the prevalence of vitamin D deficiency/ insufficiency with age but the anomalies were present even in the younger patients (table 1).

Following intervals were used for evaluation of seasonal differences: unexposed time from October to March and exposed time from April to September [29]. The winter 25-OH-D values ranged from 4.1 to 40.8 ng/mL, mean value of 21.97 ± 9.21 ng/mL, respectively the summertime 25-OH-D values ranged from 6.98 to 55.0 ng/ml, mean value of 25.03 ± 9.10 ng/mL.

DXA results in the study group

The bone mineral density, tested in the lumbar spine and in the hip, was recorded in all cases. The results are listed in table 2, for each decade. We identified normal

Age group	Number of cases	% of cases	25-OH-D (ng/ml) <u>mean±SD</u>	25-OH-D <10 ng/mL		25-OH-D <30 ng/mL	
				No	%	No	%
30-39	5	1.13	29.92±16.493	0	0	3	60
40-49	49	15.95	25.08±9.96	1	2.04	31	63.26
50-59	144	38.17	24.47±11.41	11	7.63	95	65.97
60-69	108	33.33	22.77±10.76	12	11.11	74	68.51
70-79	29	9.68	20.62±10.92	5	17.24	17	58.62
>80	6	1.70	11.98±7.05	3	50.0	3	50.0
Total	341		23.69±11.40	32	9.38	223	65.39

Table 1
AGE DISTRIBUTION OF VITAMIN D LEVELS, DEFICIENCY AND INSUFFICIENCY

DXA results				
Age group	Lumbar spine		Femoral neck	
	BMD (g/cm ²)	T score	BMD (g/cm ²)	T score
30-39	1.117±0.008	-0.45±0.727	0.898±0.082	-0.675±0.687
40-49	0.790±0.108	-1.22±1.910	0.665±0.308	-0.948±1.111
50-59	0.800±0.153	-1.748±1.693	0.691±0.108	1.231±1.061
60-69	0.773±0.106	-1.835±1.417	0.690±0.093	-1.336±0.931
70-79	0.770±0.146	-2.161±1.350	0.681±0.113	-1.721±0.913
> 80	0.903±0.196	-1.626±1.510	0.566±0.097	-2.656±0.512
Total	0.791±0.136	-1.726±1.628	0.684±0.149	-1.292±1.024

Table 2
DXA RESULTS IN DIFFERENT AGE GROUPS IN THE 341 POSTMENOPAUSAL WOMEN

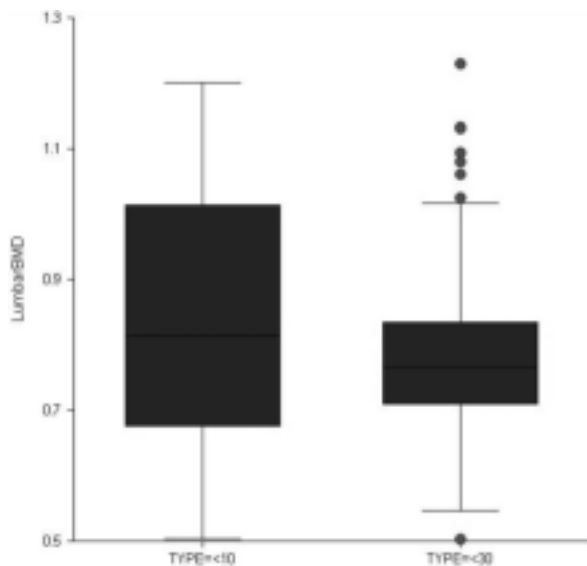


Fig. 1A. BMD (g/cm²) at lumbar spine in cases with vitamin D deficiency (32 cases) compared with BMD (g/cm²) in cases with vitamin D insufficiency (223 cases). $p = 0.567$

BMD values in 66 cases (19.35%), osteopenia in 139 cases (40.76%) and osteoporosis in 136 cases (39.88%).

The DXA measurements, revealed important differences, between BMD values at lumbar spine level, related to 25-OH-D levels, regardless of the subject's age. Surprisingly, patients with vitamin D deficiency (values lower than 10 ng/mL) had higher BMD levels than those with vitamin D insufficiency (values lower than 30 ng/mL). Both categories of patients have lower BMD values than the patients with normal vitamin D levels. The differences are not statistically significant.

At the femoral neck level, the impact of vitamin D deficiency/insufficiency is important, deficient patients having lower BMD values as compared with insufficient patients ($p=0.041$) who instead, have lower values than the normal vitamin D patients ($p=0.0001$) (fig. 2).

To evaluate the impact of vitamin D on the lumbar spine and femoral neck BMD values we built two separate multiple regression models having these two values considered as dependent variables and the following parameters considered as predictors: 25-OH-D levels, patient's age, Body mass index (BMI) and duration from the onset of menopause.

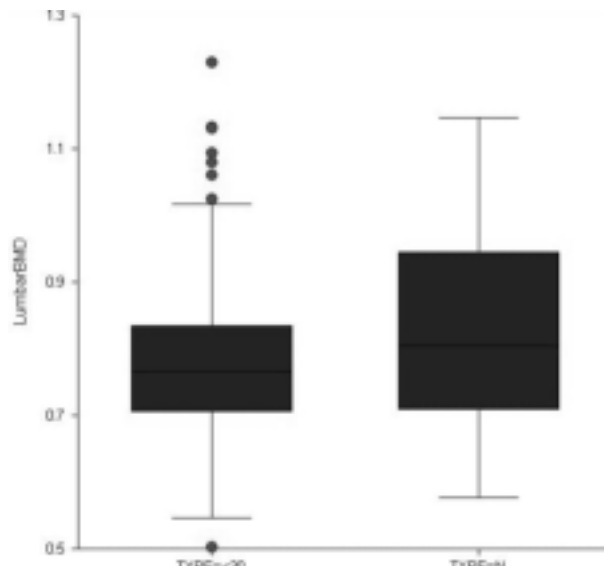


Fig. 1B. BMD (g/cm²) at lumbar spine in cases with vitamin D insufficiency (223 cases) compared with BMD (g/cm²) at lumbar spine in cases with normal levels (86 cases). $p = 0.837$

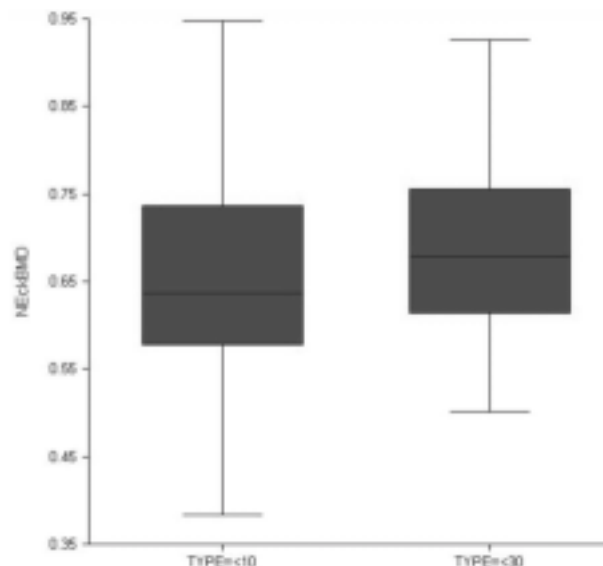


Fig. 2B. BMD (g/cm²) at the femoral neck in cases with vitamin D insufficiency (223 cases) compared with BMD (g/cm²) at the femoral neck in cases with normal levels (86 cases)

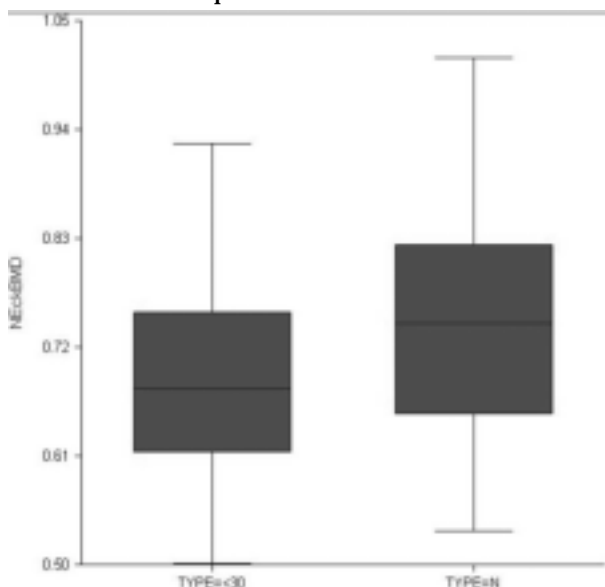


Fig. 2A. BMD (g/cm²) at the femoral neck in cases with vitamin D deficiency (32 cases) compared with BMD (g/cm²) at the femoral neck in cases with insufficiency (223 cases)

The model built for the prediction of femoral neck BMD values (g/cm²), proved to be statistically significant, observing an adjusted R² of 0.303 with a p value lower than 0.001. The results of this regression are demonstrating that the vitamin D levels are influencing also in an independent manner and not only as a confounding factor the BMD at femoral neck level. According to our model, one increase with a standard deviation in the vitamin D values is associated with an increase with 0.22 standard deviations in the BMD values (table 3).

Also, the model built for the prediction of lumbar BMD was statistically significant, observing an adjusted R² of 0.121, with a p value lower than 0.001. This model's results are leading to the conclusion that the vitamin D levels are not independently associated with the BMD lumbar values. According to our model, a significant impact on the BMD lumbar values had only the BMI of the patient, observing that for one standard deviation increase in the BMI, the lumbar BMD increased with 0.277 standard deviations (table 4).

Independent variable	B	Standardized coefficient (beta)	p
25(OH)D (ng/mL)	0.002	0.219	0.001 *
Patient's age (years)	-0.002	-0.177	0.046 *
BMI (kg/m ²)	0.010	0.411	<0.001 *
Duration from menopause onset (years)	-0.001	-0.087	0.416

* Predictor independently associated with femoral neck BMD value.

Independent variable	B	Standardized coefficient (beta)	p
25(OH)D (ng/mL)	0.001	0.081	0.249
Patient's age (years)	-0.002	-0.121	0.307
BMI (kg/m ²)	0.009	0.277	<0.001 *
Duration from menopause onset (years)	-0.001	-0.068	0.569

* Predictor independently associated with lumbar BMD value.

To evaluate the association between vitamin D levels and T scores (both lumbar and femoral neck site) we built partial correlation models between these variables, in all cases the correlations being controlled for the patient's age. These models revealed that the vitamin D levels were significantly and positively associated with both lumbar T score (Spearman's $r=0.215$; $p<0.001$) and femoral neck T score (Spearman's $r=0.333$; $p<0.001$).

ROC curve data in the study group

We analyzed 25-OH-D thresholds and their impact on bone mineral density. Using the diagnostic ROC curve and the Youden method, we tried to identify the optimal vitamin D value that is suggestive for important bone loss with low BMD values. We estimated the threshold which best predicts osteoporosis at different sites: lumbar spine,

femoral neck, or both. We obtained the following data: 25-OH-D less than 20 ng/mL predicts osteoporosis at the lumbar spine (AUC = 61.309%, $p=0.0001$) and also for the whole body (AUC = 61.893%, $p=0.0001$). 25-OH-D proved to be the best predictor for femoral neck osteoporosis (AUC = 65.678%, $p=0.001$). Based on the Youden method index J, the best diagnostic predictivity of low 25-OH-D levels is observed for identification of osteoporosis at the femoral neck level, as seen in table 5. The threshold values, with balanced sensitivity and specificity, identified with the ROC diagnostic curve method, are listed in the table 3.

There are few data regarding vitamin D status in women from Romania, this study being the first that attempts to identify a suggestive threshold of vitamin D for bone demineralization in postmenopausal women.

The results concerning the prevalence of suboptimal vitamin D levels are heterogeneous because of the use of

Table 3
MULTIPLE REGRESSION ANALYSIS RESULTS, HAVING THE FEMORAL NECK BMD CONSIDERED DEPENDENT VARIABLE

Table 4
MULTIPLE REGRESSION ANALYSIS RESULTS, HAVING THE LUMBAR BMD CONSIDERED DEPENDENT VARIABLE

Evaluation sites	25-OH-D value	Sensitivity	Specificity	J	Like hood ratio	Prev. PPV	NPV
L1-L4	21.70	58.015	62.831	0.208	1.542	49.03	70.403
	21.80	58.015	61.905	0.199	1.542	48.71	70.270
	21.90	58.015	61.429	0.194	1.504	48.40	70.109
Femoral neck	18.90	64.865	64.803	0.296	1.842	0.183	0.938
	19.20	64.865	64.474	0.293	1.825	0.181	93.75
	19.40	64.865	63.816	0.286	1.79	0.179	0.937
	20.30	64.85	64.487	0.293	1.77	0.177	0.936
Total Body	21.70	58.50	62.381	0.208	1.54	0.4903	0.704
	21.80	58.50	61.905	0.204	1.52	0.4871	0.702
	21.90	58.50	61.429	0.199	1.504	0.4848	0.701

Table 5
VITAMIN D VALUES SUGGESTIVE FOR DECREASED BMD VALUE AT LUMBAR AND FEMORAL NECK LEVEL

different cut-off levels for the definition of vitamin D deficiency/insufficiency. In this study we defined vitamin D insufficiency as levels lower than 30 ng/mL, and deficiency as levels lower than 10ng/mL. The prevalence of suboptimal vitamin D level is very high in our region (Western Romania): 72.72% of the patients presenting insufficiency, and 9.38% presenting deficiency. The data are similar to other results published in our geographical area [20].

The mean vitamin D values however are lower than the ones found in Hungary (less than 250 km distance to NW from our region): 23.69 ± 11.4 vs. 29.1 ± 10.5 [30, 31], but higher than the ones obtained in Serbia (100 km SW from our region) [32]. There are also important regional differences concerning the prevalence of vitamin D anomalies in Romania. In the SE part of the country there is a higher prevalence of vitamin D deficiency (32.2% as compared to our region from western Romania 9.38%), but lower prevalence of insufficiency (42.3% vs. 72.72%). One should note the fact that both regions have the same insolation levels (1500-1550 kWh/m²) [33]. There is no clear explanation for these differences in our country. Probably a higher number of cases are needed in order to have a clear conclusion.

In Central Europe [24] the mean wintertime 25-OH-D concentration range from around 11.2 ng/mL (28nmol/L) in Poland to 18 ng/mL (45nmol/L) in Estonia. Reported summertime 25-OH-D concentrations ranged from 18 ng/mL (45nmol/L) in Ukraine to around 35.2 ng/mL (88 nmol/L) in Hungary, and annual average values specifically in larger studies ($n > 100$) ranged from 14 ng/mL (35 mmol/L) in Ukraine to 28.8 ng/mL (72nmol/L) in Belarus.

The mean seasonal and annual 25-OH-D values obtained by us are comparable with these results, but the prevalence of vitamin D insufficiency is much higher in our country, compared with the surrounding countries. Adherence to prevention and also access to health care could be one explanation for this higher prevalence of decreased 25-OH-D values. The prevalence of vitamin D deficiency is high, in all age groups in our country and the tendency of it is to increase with age [18]. This observation is valid also for younger women (aged under 45) with surgical menopause.

The problem of vitamin D thresholds and bone health remains a controversial issue; there is no known vitamin D value that guarantees intact bone structure [34]. Histomorphometric and observational studies recommend vitamin D level over 30 ng/mL in order to avoid bone demineralization and maintain bone health [7, 35], though expert consensus recommends levels only higher than 20 ng/mL [36, 37].

BMD results showed lower values, at all sites, in patients with vitamin D deficiency or insufficiency. The decrease was more important at the femoral neck level, were the lower the 25-OH-D levels, the lower the BMD values.

Our findings suggest that 25-OH-D level is a good predictor for osteoporosis, especially at the hip level. Similar data were found in the literature, suggesting that the correlation of BMD with vitamin D is good, and also independent of age and PTH values [38, 39]. DXA measurement remains the gold standard for the diagnostic of osteoporosis and osteopenia. Nevertheless, in our country, the number and accessibility to DXA devices is reduced compared with the laboratory units. Recommending a vitamin D measurement is easy, and affordable in almost all situations. Low levels of vitamin D

suggest low mineral density and should increase the urge to recommend and perform DXA evaluation. The identified 25-OH-D value suggestive for low BMD, around 20 ng/mL, is comparable with some results from the literature [18, 36, 40-42].

Conclusions

The prevalence of suboptimal vitamin D levels is very high in the western part of our country, higher than in the southern part. 25-OH-D level is an independent predictor of femoral neck BMD value. The impact of 25-OH-D level on lumbar spine is less important. In cases with 25-OH-D values lower than 20 ng/mL urgent DXA evaluation is needed.

References

1. HEANEY, R.P., *Am. J. Clin. Nutr.* **85**, 2007, p. 300S
2. PLUDOWSKI, P., HOLICK, M.F., PILZ, S., WAGNER, C.L., HOLLIS, B.W., GRANT, W.B., SHOENFELD, Y., LERCHBAUM, E., LLEWELLYN, D.J., KIENREICH, K., SONI, M., *Autoimmunity Rev.* **12**, 2012, p. 976
3. HEANEY, R.P., *N. Engl. J. Med.* **367**, 2012, p. 77
4. ROSEN, C.J., ABRAMS, S.A., ALOIA, J.F., BRANNON, P.M., CLINTON, S.K., DURAZO-ARVIZU, R.A., GALLAGHER, J.C., GALLO, R.L., JONES, G., KOVACS, C.S., MANSON, J.E., MAYNE, S.T., ROSS, A.C., SHAPSES, S.A., TAYLOR, C.L., *J. Clin. Endocrinol. Metab.* **97**, 2012, p. 1146
5. BUSSE, B., BALE, H.A., ZIMERMANN, E.A., PANGANIBAN, B., BARTH, H.D., CARRIERO, A., VETTORAZZI, E., ZUSTIN, J., HAHN, M., Ager, J.W., PÜSCHEL, K., AMLING, M., RITCHIE, R.O., *Sci. Transl. Med.* **5**, 2013, p. 193ra88
6. RYAN, J.W., ANDERSON, P.H., TURNER, A.G., MORRIS, H.A., *Clin. Chim. Acta* **21**, 2013, p. 148
7. PRIEMEL, M., VON DOMARUS, C., KLATTE, T.O., KESSLER, S., SCHLIE, J., MEIER, S., PROKSCH, N., PASTOR, F., NETTER, C., STREICHERT, T., PÜSCHEL, K., AMLING, M., *J. Bone Miner. Res.* **2592**, 2010, p. 305
8. EASTELL, R., BARTON, I., HANNON, R.A., CHINES, A., GARNERO, P., DELMAS, P.D., *J. Bone Miner. Res.* **18**, 2003, p. 1051
9. DAWSON-HUGHES, B., HARRIS, S.S., KRALL, E.A., DALLAL, G.E., *N. Engl. J. Med.* **337**, 1997, p. 670
10. SANDERS, K.M., SCOTT, D., EBELING, P.R., *Curr. Osteoporosis Rep.* **12**, 2014, p. 74
11. REID, I.R., BOLLAND, M.J., *Osteoporosis Int.* **25**, 2014, p. 2347
12. PERETIANU, D., GRIGORIE, D., ONOSE, G., *Rom. Acad. Publishing House*, 2002, p. 166
13. HILL, T.R., ASPRAY, T.J., FRANCIS, R.M., *Proc. Nutr. Soc.* **72**, 2013, p. 372
14. GALLAGHER, J.C., *Endocrinol. Metab. Clin. North Am.* **42**, 2013, p. 319
15. MACLAUGHLIN, J., HOLICK, M.F., *J. Clin. Invest.* **76**, 1985, p. 1536
16. TSAI, K.S., HEATH, H., KUMAR, R., RIGGS, B.L., *J. Clin. Invest.* **73**, 1984, p. 1668
17. BAILEY, R.L., DODD, K.W., GOLDMAN, J.A., GAHCHE, J.J., DWYER, J.T., MOSHFEGH, A.J., SEMPOS, C.T., PICCIANO, M.F., *J. Nutr.* **140**, 2010, p. 817
18. GRIGORIE, D., NEACSU, E., MARINESCU, M., DUMITRACHE, L., POPA, O., *Acta Endocrinol. (Buc)* **1**, 2005, p. 369
19. POROJNICU, A.C., MOROTI-CONSTANTINESCU, R., LASLAU, A., LAGUNOVA, Z., DAHLBACK, A., HRISTEA, A., MOAN, J., *Public Health Nutr.* **15**, 2012, p. 2157
20. PLUDOWSKI, P., GRANT, W., BHATTOA, H., BAYER, M., POVORODNYUK, V., RUDENKA, E., RAMANAU, H., VARBIRO, S., RUDENKA, A., KARCZMAREWICZ, E., LORENC, R., CZECH-KOWALSKA, J., KONSTANTYNOWICZ, J., *Int. J. Endocrinol.* 2014, p. 589587
21. SPIRO, A., BUTTRISS, J.L., *British Nutr. Foundation* **39**, 2014, p. 322
22. SACN (Scientific Advisory Committee on Nutrition). Update on vitamin D. Position statement. London: The Stationary Office, 2007
23. *** Cancer Research UK. Available at: <http://www.nice.org.uk/nicemedia/live/11871/49665/49665.pdf>

24. PLUDOWSKI, P., KARCZMAREWICS, E., BAYER, M., CARTER, G., CHLEBNA-SOKOL, D., CZECH-KOWALSKA, J., DEBSKI, R., DECSI, T., DOBRZANSKA, A., FRANEK, E., GLUSZKO, P., GRANT, W.B., HOLICK, M.F., YANKOVSKAYA, L., KONSTANTYNOWICZ, J., KSIAZYK, J.B., KSIEZOPOLSKA-ORLOWSKA, K., LEWINSKI, A., LITWIN, M., LOHNER, S., LORENC, R.S., LUKASZKIEWICZ, J., MARCINOWSKA-SUCHOWIERSKA, E., MILEWICZ, A., MISIOROWSKI, W., NOWICKI, M., POVOROZNYUK, V., ROZENTRYT, P., RUDENKA, E., SHOENFELD, Y., SOCHA, P., SOLNICA, B., SZALECKI, M., TALALAJ, M., VARBIRO, S., Z-MIJEWSKI, M.A., *Endokrinol. Polska* **64**, 2013, p. 319
25. CHAPUY, M.C., PREZIOSI, P., MAAMER, M., ARNAUD, S., GALAN, P., HERCBERG, S., MEUNIER, P.J., *Osteoporos Int.* **7**, 1997, p. 439
26. HEANEY, R.P., *Am. J. Clin. Nutr.* **80**, 2004, p. 1706
27. ALOIA, J.F., *J. Clin. Endocrinol. Metab.* **96**, 2011, p. 2987
28. HOLICK, M.F., BINKLEY, N.C., BISCHOFF-FERRARI, H.A., GORDON, C.M., HANLEY, D.A., HEANEY, R.P., MURAD, M.H., WEAVER, C.M., *Endocrine Soc., JCEM* **96**, 2011, p. 1911
29. O'CONNOR, A., BENELAM, B., *Nutr. Bull.* **36**, 2011, p. 390
30. VASARHELYI, A., SATORI, F., OLAJOS, A., SZABÓ, A., BEKO, G., *Orvosi Hetilap* **152**, 2011, p. 1272
31. BHATTOA, H.P., BETTMBUK, P., GANACHARYA, S., BALOGH, A., *Osteoporosis Int.* **15**, 2004, p. 447
32. JOVICIC, S., IGNJATOVIC, S., KANGRGA, R., BELETIÆ, A., MIRKOVIÆ, D., MAJKIC-SINGH, N., *J. Med. Biochim.* **31**, 2012, p. 347
33. SURI, M., HULD, T.A., DUNLOP, E.D., OSSENBRINK, H.A., *Solar Energy* **81**, 2007, p. 1295
34. BOULLION, R., BISCHOFF-FERRARI, H., WILLER, W., *J. Bone Min. Res.* **23**, 2008, p. 974
35. DAWSON-HUGHES, B., HEANEY, R., HOLICK, M.F., LIPS, P., MEUNIER, P.J., VIETH, R., *Osteoporosis Int.* **16**, 2005, p. 713
36. RIZZOLI, R., BOONEN, S., BRANDI, M.L., BRUYÈRE, O., COOPER, C., KANIS, J.A., KAUFMAN, J.M., RINGE, J.D., WERYHA, G., REGINSTER, J.Y., *Curr. Med. Res. Opin.* **29**, 2013, p. 305
37. ROUX, C., BISCHOFF-FERRARI, H.A., PAPAPOULOS, S.E., DE PAPP, A.E., WEST, J.A., BOUILLON, R., *Curr. Med. Res. Opin.* **24**, 2008, p. 1363
38. MALAVOLTA, N., PRATELLI, L., FRIGATO, M., MULE, R., MASCIA, M.L., GNUDI, S., *Osteoporosis Int.* **16**, 2005, p. 1691
39. ARABI, A., BADDOURA, R., AWADA, H., SALAMOUN, M., AYOUB, G., EL-HAJJ FULEIHAN, G., *Bone* **39**, 2006, p. 268
40. MALABANAN, A., VERONIKIS, I.E., HOLICK, M.F., *Lancet.* **351**, 1998, p. 805
41. CRANNEY, A., HORSLEY, T., O'DONNELL, S., WEILER, H., PUIL, L., OOI, D., ATKINSON, S., WARD, L., MOHER, D., HANLEY, D., FANG, M., YAZDI, F., GARRITTY, C., SAMPSON, M., BARROWMAN, N., TSERTSVADZE, A., MAMALADZE, V., *Evid. Rep. Technol. Assess (Full Rep.)* **158**, 2007, p. 1
42. GRIGORIE, D., NEACSU, E., BARBU, C., DUMITRACHE C., *Rev. Rom. Osteoporoză* **4**, 2000, p. 36

Manuscript received: 22.08.2015