

CLINICAL STUDY

Vitamin D status in apparently healthy medication-free Slovaks: Association to blood pressure, body mass index, self-reported smoking status and physical activity

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ABSTRACT

BACKGROUND: Vitamin D plays a role in protecting against chronic degenerative diseases. Slovak adults present one of the highest cardiovascular mortality rates among 27 EU countries.

OBJECTIVES: We asked whether the 25(OH)D₃ status in apparently healthy medication-free Slovaks deteriorates upon ageing, and in the presence of cardiometabolic risk factors.

METHODS: We studied the impact of blood pressure, overweight/obesity, smoking, and physical activity on 25(OH)D₃ levels determined using RIA method in 578 (5–81 years old) subjects.

RESULTS: The average level of 25(OH)D₃ was 36±17 ng/ml. A proportion of 15 % of participants were 25(OH)D₃-deficient (≤20 ng/ml), 26 % presented insufficient (20-to-30 ng/ml), and 59 % satisfactory (> 30 ng/ml) levels. Neither mean 25(OH)D₃ levels, nor the prevalence of hypovitaminosis D showed age dependence. Physically active normotensive non-smokers presented the highest (41±19 ng/ml), and their smoking counterparts with elevated BP the lowest 25(OH)D₃ levels (30±12 ng/ml).

CONCLUSION: In apparently healthy medication-free Slovaks the prevalence of hypovitaminosis D is high. Vitamin D status does not deteriorate in course of healthy ageing. Physical activity, normotension, and non-smoking status are associated with favorable vitamin D status while low 25(OH)D₃ levels are associated with multiple cardiometabolic risk factors. Further studies in subjects at high cardiovascular risk are needed to elucidate the potential association of hypovitaminosis D with high cardiovascular mortality in Slovak adults (Tab. 1, Fig. 4, Ref. 42). Text in PDF www.elis.sk.

KEY WORDS: vitamin D, blood pressure, smoking, physical activity, body mass index.

Introduction

Vitamin D is a fat-soluble steroid, formed in the dermis from 7-dehydrocholesterol via ultraviolet irradiation. Provitamin D₃ quickly undergoes photochemical and thermal rearrangements to form a stable vitamin D₃. After binding to vitamin D-binding protein (DBP), it enters circulation and is hydroxylated first in the

liver to form 25(OH)D₃, and thereafter in the kidneys to produce the active vitamin in form of 1,25(OH)₂D₃.

The effects of active vitamin D are mediated predominantly by binding to its nuclear receptor (VDR). Together with other transcription factors such as retinoid X receptor (RXR), the complex of vitamin D, VDR and RXR is formed and bound to vitamin D response elements of multiple genes, regulating directly and/or indirectly their expression (1–3).

The effects of vitamin D on skeleton, its role in proper hormone secretion, and functioning of neural transmission and neuromuscular junctions have been well known for a long time (2, 4, 5).

Recently, it has been recognized that 1,25(OH)₂D₃ is produced locally in a wide variety of extrarenal cells expressing VDR. It is presumed that local production of vitamin D is responsible for extraskeletal modulation of various physiological processes with a series of adverse consequences associated with inadequate 1,25(OH)₂D₃ production, such as increased risk of hypertension, insulin resistance, type 1 and 2 diabetes, and different cancers (2, 6). Current evidence suggests that 25(OH)D₃ levels above 30 ng/ml may potentially exert non-calcemic health benefits. As documented in the large prospective study, inadequate vitamin D levels (< 15 ng/ml) represent an important cardiovascular (CV) risk factor and might play a causal role in the development of CV risk factors,

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CV diseases and adverse events including death (7). An increased CV mortality of vitamin D-deficient subjects was confirmed also by the recent meta-analysis (8). A significant relationship between vitamin D deficiency and cardiometabolic risk factors has been documented even in obese children and adolescents (9).

Among EU countries, the prevalence of overweight, obesity and hypertension are highest in Slovakia, and they associate with unfavorable nutrition, smoking, and a lack of physical activity (10–12). The high prevalence of CV disease risk factors is reflected by one of the highest CV mortality rates of Slovak adults among 27 EU countries (11, 13).

We posed a question whether in apparently healthy medication-free Slovaks, the vitamin D status deteriorates by ageing, and how it is associated with CV risk factors (BP, overweight/obesity, physical activity, smoking status), and their combination.

Subjects and methods

This study was conducted according to Declaration of Helsinki, after the approval of the protocols by ethics boards of SMU in Bratislava and Medical Faculty of UJPS in Košice. Participating subjects or their parents (for under-aged) signed written informed consent to participate.

Subjects

Apparently healthy medication-free volunteers of Central European descent attending their regular preventive examination were recruited via general practitioners, or using advertisements posted in frequented public locations in two Slovak cities with similar geographic latitude: Bratislava: 48.1°N, 17.0°E, and Košice: 48.4°N, 21.1°E. Members of minorities with darker skin where not included (14). Further exclusion criteria were age < 5 years, any acute and/or chronic diseases, unstable health conditions, and pregnancy and lactation in women. Subjects declaring that they were not supplemented by vitamin D (including any over-the-counter vitamin D supplements) during at least last six months underwent complete medical examination by a physician at outpatients departments prior to enrollment.

Measurements

Body weight was measured using calibrated electronic balance; height was measured using stadiometer. Body mass index (BMI) was calculated. Adults presenting BMI ≤ 24.9 kg/m², and children and adolescents in BMI < 90th percentile for their gender and age (15) according to 1991 Slovak charts were considered having normal body weight. Blood pressure readings were taken in the right forearm in sitting subjects resting for at least 10 minutes, using standard sphygmomanometer. The mean of last two readings out of three taken was considered. In adults, systolic BP (SBP) ≥ 130 mm Hg and/or diastolic BP (DBP) ≥ 85 mm Hg was considered to be a risk factor for cardiometabolic disease (16). In children and adolescents, BP > 90th percentile for gender, height and age was considered elevated (17).

Blood was sampled after overnight fasting. Serum was stored at -80 °C till analysis. Concentrations of 25(OH)D₃ were deter-

mined by RIA method (25(OH)D₃, Immuno Diagnostic system, Boldon, UK) at SMU. Intra- and inter-batch coefficients of variation were < 10 %. The state of 25(OH)D₃ deficiency was considered at concentrations ≤ 20 ng/ml; insufficiency was considered at levels ranging between 20 and 30 ng/ml, while higher levels were considered satisfactory (5).

Self-reported current smoking status was recorded as “yes” or “no”. Subjects reporting physical activity for longer than 30 min/day at least 3 times per week were classified as physically active; those not exercising at all or reporting less frequent shorter physical activity were classified as sedentary. Physical activity comprehended exercising, cycling, dancing, gardening, etc.

Statistical analyses

Data not matching the normal distribution were logarithmically (ln) transformed for statistical evaluation, but for better understanding, mean \pm standard deviation ($x \pm SD$) are given. Means of two groups were compared using Student's t-test; three groups were compared using analysis of variance (ANOVA) with post-hoc Scheffé's test, if appropriate. Chi-square test (with Yates' correction where appropriate) was used to compare categorical data. Pearson's or Spearman's correlation coefficients were calculated.

Tab. 1. Cohort characteristics and 25(OH)D₃ levels with regards to smoking status, body weight, physical activity and blood pressure in whole cohort, males and females.

	All	Males	Females	p
N (%)	578	254 (44%)	324 (56%)	–
Age (years)	33.81 \pm 14.7	34.0 \pm 15.0	33.7 \pm 14.5	0.81
BMI (kg/m ²)	24.8 \pm 5.0	26.3 \pm 5.0	23.7 \pm 4.7	<0.001
SBP (mm Hg)	123 \pm 14	128 \pm 14	120 \pm 13	<0.001
DBP (mm Hg)	76 \pm 9	77 \pm 9	75 \pm 9	0.003
Vitamin D concentrations				
25(OH)D ₃ (ng/ml)	35.6 \pm 16.8	35.1 \pm 17.2	36.0 \pm 16.4	0.48
Smoking status				
Current non-smokers	36.6 \pm 17.0	36.1 \pm 17.2	37.0 \pm 17.0	0.76
Current smokers	34.0 \pm 16.4	33.6 \pm 18.4	34.3 \pm 14.8	0.42
p	0.11	0.14	0.40	–
Body mass index				
Normal	35.7 \pm 16.8	34.8 \pm 17.1	36.3 \pm 16.6	0.29
Overweight/obese	35.2 \pm 16.4	36.4 \pm 17.7	33.3 \pm 14.3	0.48
p	0.80	0.55	0.35	–
Physical activity				
Physically active	38.0 \pm 17.0	37.0 \pm 17.8	38.8 \pm 16.4	0.26
Sedentary	33.4 \pm 16.4	33.1 \pm 16.8	33.5 \pm 16.2	0.78
p	<0.001	0.045	0.002	–
Blood pressure				
Normotensive subjects	36.3 \pm 16.7	36.5 \pm 18.8	36.2 \pm 15.5	0.84
Elevated blood pressure	34.51 \pm 16.9	33.9 \pm 15.6	35.4 \pm 18.8	0.69
p	0.22	0.37	0.53	–

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; 25(OH)D₃: 25-hydroxy vitamin D₃; chi: chi-square; italics: statistical evaluation performed on logarithmically transformed data; normal weight classified as BMI < 95th percentile for age and gender in children and adolescents, and BMI ≤ 24.9 kg/m² in adults; overweight/obesity classified as BMI ≥ 95 th percentile for age and gender in children and adolescents, and BMI ≥ 25.0 kg/m² in adults; subjects reporting physical activity lasting at least 30 min for at least 3 times per week were classified as physically active; subjects not exercising at all or reporting less frequent and shorter physical activity were classified as sedentary; elevated blood pressure was classified as SBP ≥ 130 mm Hg and/or DBP ≥ 85 mm Hg in adults and blood pressure > 95th percentile for gender, height and age in children and adolescents

Multivariate analysis employing General linear model (GLM) and classification tree with season of blood sampling entered as an influence variable were employed to study the impact of independent variables on 25(OH)D₃ levels. Only subjects aged ≥18 years in whom data on all risk factors were available were included. The value of $p < 0.05$ (2-sided) was considered significant. Statistical analyses were performed using program SPSS version 22 (SPSS Inc., Chicago, IL, USA). In addition, software for orthogonal projections to latent structures analysis (OPLS, Simca v.14.1 software, Umetrics, Umea, Sweden) was used to identify the predictors of 25(OH)D₃ levels. Variables with variable of importance for the projection (VIP) values > 1 were considered to be important contributors, while those with $VIP < 0.5$ were considered unimportant. VIP values in range of 0.5–1 are generally considered to be in “grey interval”; the importance of variable depends on the sample size.

Results

General characteristics

Out of 589 recruited subjects, 11 in whom the 25(OH)D₃ levels were not determined for technical reasons were not included into this analysis. Thus, the cohort consisted of 578 apparently healthy subjects aged 5–81 years; characteristics are given in Table 1. A proportion of 38 % of subjects presented elevated BP with higher prevalence among males (53 %) in comparison with females (27 %, $p_{\text{chi}} < 0.001$). Subjects with elevated BP were older than their normotensive counterparts (42.1 ± 15.6 vs 28.6 ± 11.3 years; $p > 0.001$). A proportion of 18 % of participants were overweight or obese with higher prevalence among males (24 %) than among females (12 %; $p_{\text{chi}} < 0.001$). Those presenting overweight/obesity were older than their lean counterparts (43.9 ± 15.8 vs 31.6 ± 13.4 years; $p > 0.001$). Data on smoking status were not available from 31 children/adolescents and 7 adults. Out of 540 subjects evaluated for smoking status, 77 % were current non-smokers, while the prevalence of non-smokers did not differ between genders (77 % vs 77 %, $p_{\text{chi}} = 0.97$). Smokers and non-smokers were of similar age (34.8 ± 13.1 vs 34.1 ± 15.1 years; $p = 0.64$). Thirty-eight children/adolescents were not included into the analysis on the effects of physical exercise. Out of 540 evaluated subjects, 57 % were exercising regularly; sedentary life style was less frequent among males (38 %) in comparison with females (47 %; $p_{\text{chi}} = 0.033$). Physically active and sedentary subjects were of similar age (38.0 ± 17.0 vs 33.4 ± 16.4 years; $p = 0.31$).

25(OH)D₃ levels

Seasonal variation

25(OH)D₃ levels ranged between 2.5 ng/ml and 141.7 ng/ml. The annual average was 35.6 ± 16.8 ng/ml without significant difference between males and females (Tab. 1). Variation in mean levels throughout the year is given in Figure 1a. Eighty-seven participants (15 %) were 25(OH)D₃-deficient with 25(OH)D₃ levels averaging 15.3 ± 3.6 ng/ml, while 151 subjects (26 %) presented insufficient (25.1 ± 2.8 ng/ml) and 59 % satisfactory 25(OH)D₃ levels (45.5 ± 14.8 ng/ml). The prevalence of 25(OH)D₃ deficiency, insufficiency, and satisfactory levels throughout the year are given in Figure 1b.

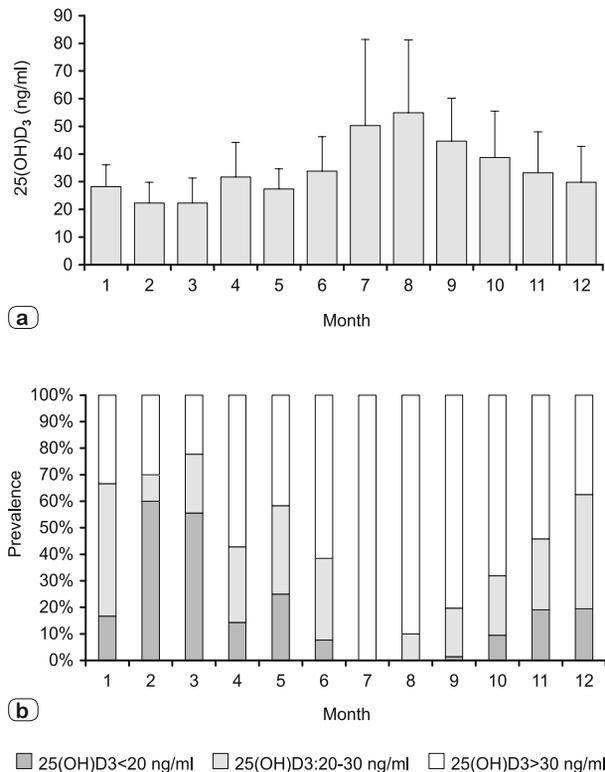


Fig. 1. Seasonal variation in 25(OH)D₃ levels and in prevalence of 25(OH)D₃ deficiency (≤20 ng/ml), insufficiency (25(OH)D₃ concentration in range 20–30 ng/ml), and satisfactory levels (25(OH)D₃ >30 ng/ml). 1a: Seasonal variation in mean 25(OH)D₃ levels, 1b: Seasonal variation in the prevalence of 25(OH)D₃ deficiency, insufficiency and satisfactory levels 1: January; 2: February; 3: March; 4: April; 5: May; 6: June; 7: July; 8: August; 9: September; 10: October; 11: November; 12: December.

Effects of ageing

Mean 25(OH)D₃ levels were similar among age groups ($p_{\text{ANOVA}} = 0.48$) (Fig. 2a). Neither the prevalence of 25(OH)D₃ insufficiency, deficiency or satisfactory levels differed significantly (Fig. 2b) ($p_{\text{chi-Yates}} = 0.76$).

Effect of single risk factor

Current smoking status or presence of overweight/obesity did not affect 25(OH)D₃ levels significantly in the whole cohort, either in males or in females (Tab. 1). Significant relationship between \ln 25(OH)D₃ and BMI was revealed only in overweight/obese subjects ($r = -0.209$; $p = 0.035$). The prevalence of 25(OH)D₃ deficiency, insufficiency, and satisfactory levels was similar in overweight/obese vs. lean subjects ($p_{\text{chi}} = 0.55$) as well as in smokers vs. nonsmokers ($p_{\text{chi}} = 0.28$). Although our subjects presenting elevated vs. normal BP did not differ in 25(OH)D₃ levels significantly (Tab. 1), the prevalence of 25(OH)D₃ deficiency, insufficiency and satisfactory levels differed significantly (normotensive subjects: 12 %, 25 % and 63 %, respectively; subjects with elevated blood pressure: 20 %, 27 %, and 53 %, respectively; $p_{\text{chi}} = 0.018$). Physically active subjects presented significantly higher 25(OH)D₃ lev-

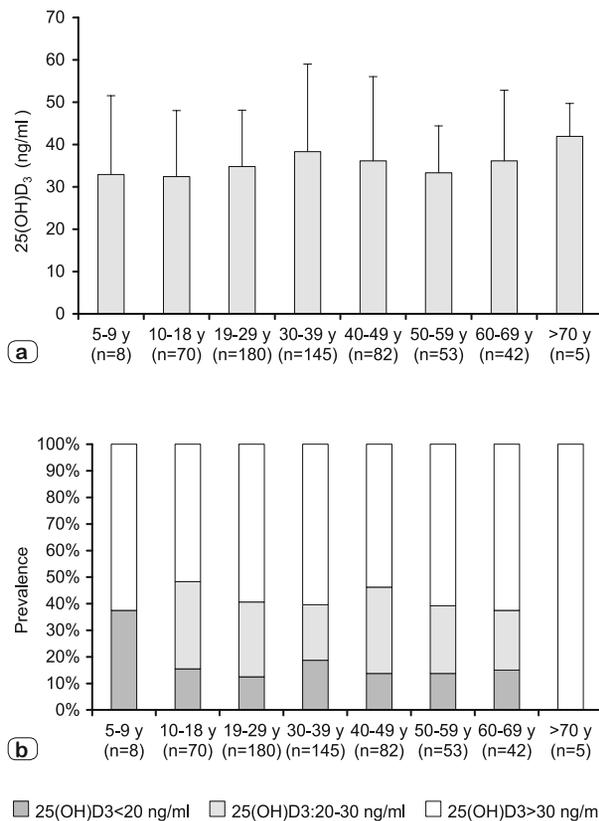


Fig. 2. Serum 25(OH)D₃ concentrations and prevalence of 25(OH)D₃ deficiency (≤ 20 ng/ml), insufficiency (25(OH)D₃ concentration in range 20–30 ng/ml), and satisfactory levels (25(OH)D₃ > 30 ng/ml) according to age categories. 2a: Mean 25(OH)D₃ concentrations according to age categories ($p_{ANOVA} = 0.48$), 2b: Prevalence of 25(OH)D₃ deficiency, insufficiency and satisfactory levels in different age categories, y: years.

els in comparison with their sedentary counterparts both in males and females (Tab. 1). Higher levels in physically active subjects were evident throughout the year, reaching significance in period from October-to-March (Fig. 3). The prevalence of 25(OH)D₃ deficiency, insufficiency, and satisfactory levels differed significantly between the sedentary participants (19 %, 29 % and 52 %, respectively) in comparison with their exercising counterparts (11 %, 24 % and 65 %, respectively; $p_{chi} = 0.006$). The prevalence of overweight/obesity was significantly higher among sedentary vs physically active subjects (25 % vs 12.3 %; $p_{chi} > 0.001$), while that of elevated BP (42.2 % vs 37.7 %; $p = 0.28$) and active smokers (26.3 % vs 21.8 %; $p = 0.22$) did not differ significantly.

Interaction of risk factors

GLM and OPLS models were used to test the effects of presence/absence of the risk factors on 25(OH)D₃ levels. OPLS indicated that the risk factors as independent predictors explain only 3% in the variability of 25(OH)D₃. The presence of physical activity and leanness was associated significantly with higher 25(OH)D₃ levels (VIP values 1.47 and 1.03, respectively). GLM selected interactions as being significant as follows: interactions between

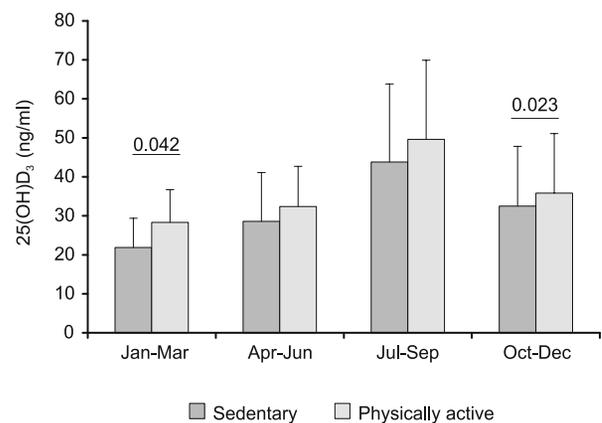


Fig. 3. Seasonal variation in serum 25(OH)D₃ levels in sedentary and physically active adults. Sed.: sedentary subjects; PA: physically active subjects, e.g. exercising at least 3 times a week for minimally 30 min; Jan–Mar: January-to-March; Apr–Jun: April-to-June; Jul–Sep: July-to-September; Oct–Dec: October-to-December.

presence/absence of physical activity, overweight/obesity, and smoking (overall model $p = 0.024$; interaction between physical activity and smoking: $p = 0.029$; physically active non-smokers: beta: -69 , SE: 0.32; interaction between physical activity, overweight/obesity and smoking: $p = 0.020$; physically active lean non-smokers: beta: -0.89 , SE: 0.38; $R^2 = 0.025$). The effect of interaction of the risk factors was further analyzed employing the answer tree with season of blood sampling used as an influence variable.

The model selected physical activity as the main determinant of 25(OH)D₃ levels (Fig. 4), confirming higher 25(OH)D₃ levels in physically active subjects vs. their sedentary counterparts.

In physically active subjects, BP was the second influencing risk factor while elevated BP was associated with lower 25(OH)D₃ levels. In the next nodes, the model designated the smoking status as the influencing variable in both BP categories of physically active subjects with lower 25(OH)D₃ levels in current smokers.

In sedentary subjects, the model denoted overweight/obesity as the determinant of lower 25(OH)D₃ levels. Further analysis confirmed that smoking (non-smokers: $n = 171$, 33.5 ± 15.9 ; smokers: $n = 61$, 33.0 ± 17.9 ng/ml, $p = 0.65$) or elevated BP (normotension: $n = 134$, 33.6 ± 14.8 ; elevated BP: $n = 98$, 33.0 ± 18.5 ng/ml, $p = 0.57$) were associated with a tendency towards lower 25(OH)D₃ levels even among sedentary subjects. Physically active normotensive non-smokers presented the highest levels, while the physically active smokers with elevated BP and overweight/obese sedentary subjects had the lowest 25(OH)D₃ levels (Fig. 4). Forcing the gender as an independent variable into the model did not affect the results.

Discussion

Apparently healthy medication-free Slovaks presented commonly low 25(OH)D₃ levels and a high prevalence of hypovitaminosis D, regardless of their age. Physical activity appeared to be the main determinant of favorable vitamin D status. Clustering of

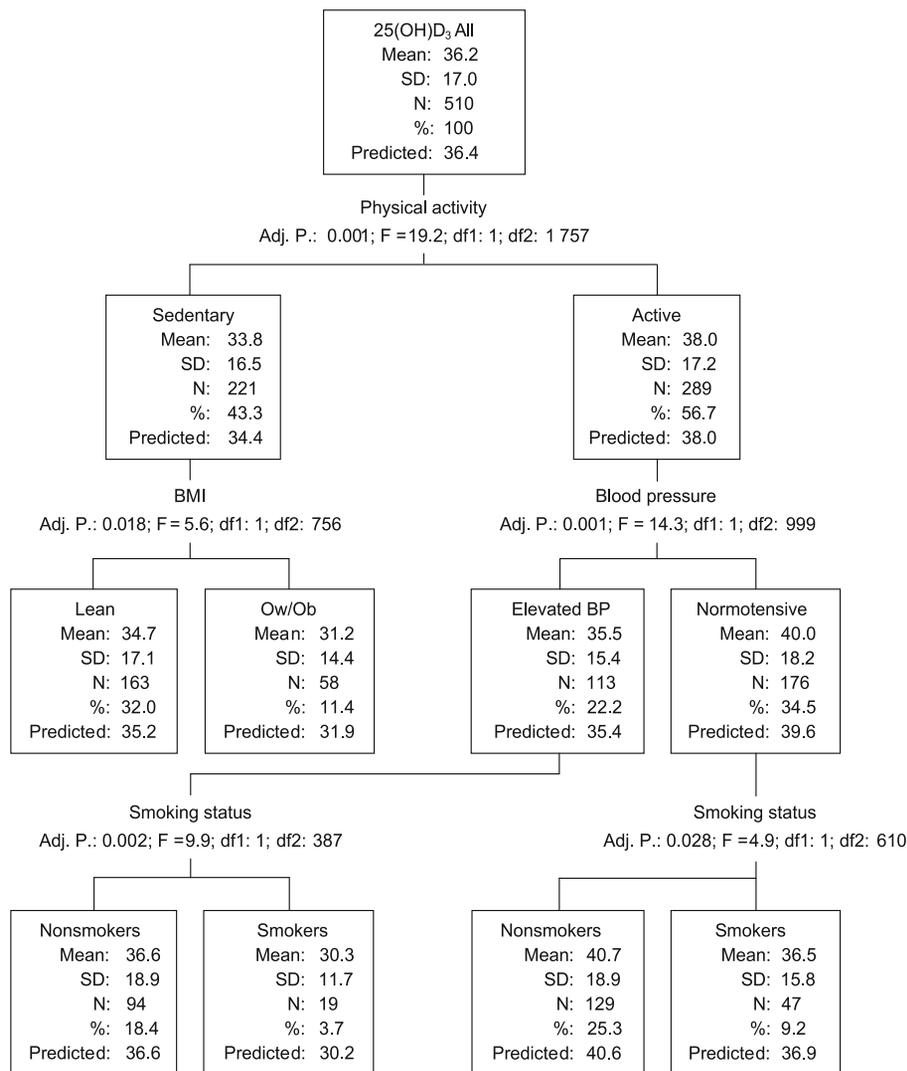


Fig. 4. Decision tree – impact of physical activity, overweight/obesity, blood pressure and smoking status on 25(OH)D₃ levels in adults (≥ 19 years). Std. Dev.: standard deviation; Adj.: adjusted; BMI: body mass index; Lean: BMI ≤ 24.9 kg/m²; Ow/ob: overweight and obese, BMI > 25.0 kg/m²; BP: blood pressure; elevated blood pressure classified as systolic BP ≥ 130 mm Hg and/or diastolic BP ≥ 85 mm Hg; normotension classified as systolic BP < 130 mm Hg and/or diastolic BP < 85 mm Hg.

risk factors, rather than the presence of a single risk factor *per se*, was associated with unfavorable vitamin D status. However, the studied risk factors (e.g. sedentary life style, overweight/obesity, elevated BP and current smoking) explained only minor variability in 25(OH)D₃ levels.

25(OH)D₃ status

As follows from the recent review on vitamin D status in Central Europe (18), only 2 studies in general adult population from countries neighboring Slovakia (i.e. Czech Republic, Hungary, Poland, Ukraine) reported mean levels of 25(OH)D₃ < 30 ng/ml (19, 20). Data on vitamin D status in general Slovak population are not available. Another Slovak study tackling vitamin D status in apparently healthy subjects (pre-

menopausal 25–40 years old women with normal bone mineral density) reported similar mean 25(OH)D₃ levels (33 ± 13 ng/ml), as well as a similar prevalence of satisfactory 25(OH)D₃ levels (51 %) to those observed in our study (21). On the other hand, small clinical studies (on type 1 diabetic children, patients with Down syndrome, patients in stages 2–3 of chronic kidney disease, patients with pulmonary sarcoidosis, post-menopausal women with osteopenia/osteoporosis, or elderly subjects with metabolic syndrome) reported median 25(OH)D₃ levels in range 19–30 ng/ml, and low prevalence of satisfactory levels (9–36 %) (22–27). Thus, it might be expected that in general Slovak population, mean 25(OH)D₃ levels as well as the prevalence of satisfactory 25(OH)D₃ status would be much lower than those observed in apparently healthy volunteers. Further

larger studies tackling general population and clinical cohorts are definitely needed to objectify vitamin D status in the Slovaks, and its potential association with morbidity and mortality, particularly from CV diseases and cancer.

25(OH)D₃ and physical activity

In our cohort, physical activity appeared to be the main determinant of 25(OH)D₃ status. The differences between physically active and sedentary subjects were present regardless of the season of blood sampling, especially during winter and early spring. This finding is of particular importance, since in Eurobarometer 2013, only 34 % of Slovaks declared that they exercise regularly or with some regularity. It remains unclear whether physical activity independent of sun exposure plays a role in modulating 25(OH)D₃ levels, or healthy lifestyle of physically active subjects denotes also higher dietary intake of vitamin D from natural sources. In Slovakia, foods are not fortified with vitamin D. However, we tracked neither the question whether exercising was performed indoors or outdoors, nor followed the dietary intake of vitamin D. Higher 25(OH)D₃ levels in physically active subjects might also reflect the lower prevalence of overweight/obesity in this cohort. Our data also document that beneficial effects of physical activity on vitamin D status might not completely counteract the negative influence of other risk factors such as elevated BP, and particularly smoking. Similar interactions might underlie discrepant results in studies on 25(OH)D₃ levels in subjects practicing regular physical activity in comparison with their sedentary counterparts (28–31).

25(OH)D₃ and blood pressure

Vitamin D may contribute to BP control via modulation of different metabolic pathways, such as absorption of calcium from the gut; effects on the renin-angiotensin system, endothelial function and arterial stiffness, or development of insulin resistance predisposing to hypertension (32–34). Large cross-sectional observational studies have linked low 25(OH)D₃ levels with increased BP (35). On the other hand, the antihypertensive effects of vitamin D supplementation are ambiguous (36, 37). In our study, normotension was generally associated with a tendency towards higher 25(OH)D₃ levels, but elevated BP showed a significant negative impact on 25(OH)D₃ levels only in physically active subjects. The reasons are unclear, but our findings indicate that in apparently healthy subjects, several other factors modulate the association of 25(OH)D₃ levels with BP.

25(OH)D₃ and overweight/obesity

The current evidence links overweight and obesity to poorer vitamin D status (38, 39). However, the overall strength of the consistent inverse association between overweight/obesity and low vitamin D status is usually weak (38). Potential mechanisms suggest sequestration of 25(OH)D₃ in adipose tissue, volumetric dilution, negative feedback mechanisms from increased circulating 1,25-dihydroxyvitamin D₃, or decreased endogenous production of cholecalciferol in the skin due to lesser outdoor activities of heavier individuals potentially wearing more clothing (38). In

our study overweight/obesity was associated with lower 25(OH)D₃ levels only in sedentary subjects. On one hand, this might reflect the higher prevalence of overweight/obesity in sedentary subjects. On the other hand, the contribution of life-style factors such as sun exposure, dietary vitamin D intake, and other ones not tracked in our study cannot be excluded.

25(OH)D₃ and smoking

In large cross-sectional studies, smoking is generally but not unequivocally, associated with lower mean 25(OH)D₃ levels or higher prevalence of vitamin D deficiency (29, 30, 40, 41). The exact mechanisms by which the constituents of tobacco smoke affect vitamin D metabolism are still unclear, but adverse effects on the level of 25(OH)D₃ synthesis are anticipated (42). In smokers, less sun exposure due to shorter outdoor activities might also not be excluded (41). Although in our study, smoking was significantly associated with lower 25(OH)D₃ levels only in physically active subjects, the tendency towards lower levels was observed also in their sedentary counterparts. However, we neither stratified for daily number of smoked cigarettes or for passive exposure to cigarette smoke, nor recorded potential smoking cessation during last few months. We also might speculate that in apparently healthy subjects, the sedentary life style *per se* is the major determinant of vitamin D status. Thus, our finding requires further targeted studies.

Strengths and limitations

This is the first study in Slovakia on vitamin D status in apparently healthy subjects of both genders and in a wide age range – from childhood to the elderly. Elderly subjects were not institutionalized. As our volunteers were virtually all of Central European descent, residing in Bratislava or Košice region, the inter-subject variability in regard to sun exposure and darkness of the skin is likely to be small. However, our volunteers were not completely unrelated, i.e. close relatives have participated. Our data on elevated blood pressure might be partially elusive because they were based on single measurements in subjects declaring that they had not been diagnosed and treated for hypertension. Voluntary participation and inclusion of apparently healthy medication-free subjects indicate that our data reflect the status in an “overhealthy” population. The number of enrolled subjects aged over 60 years was low, reflecting that seniors generally suffer from different diseases and are on medication. Another limitation is that we used simple self-report to quantify physical activity and smoking status. Physical activity was not tracked in pre-school children and those attending primary schools. In the latter ones, due to the fact that pupils attending schools providing > 2 classes of gym per week would automatically classify as physically active. Missing data and (as expected) low prevalence of risk factors in children/adolescents did not allow for separate analysis of associations in this cohort. Moreover, the cross-sectional nature of the study requires caution in interpreting the associations delineated. Additional limitations are discussed above in relation to pertinent subject.

Conclusion

Our cross-sectional data suggest that in apparently healthy subjects, vitamin D status does not deteriorate in the course of healthy ageing, and underline the association of physical activity, absence of overweight/obesity, and non-smoking status with satisfactory vitamin D levels. Unsatisfactory vitamin D status is associated with the clustering of cardiovascular risk factors. However, the investigated risk factors explained only a minor proportion in the variability of 25(OH)D₃ levels. Thus, whether the introduction of regular physical activity, reduction of body fatness, and smoking cessation could effectively improve vitamin D status at least for subjects presenting a particular clustering of cardiovascular risk factors remains to be elucidated.

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