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RELATION OF SERUM 25(OH)-VITAMIN D TO FAT MASS AND LEAN MASS, BONE MINERAL CONTENT AND BODY COMPOSITION IN ADULTS – A DXA-BASED STUDY

M. G. Nikolova[1], A. B. Penkov[1], M. A. Boyanov[2]

[1]Department of Hygiene, Medical Ecology and Nutrition, Faculty of Medicine, Medical University – Sofia, Bulgaria
[2]Clinic of Endocrinology and Metabolic Diseases, University Hospital "Alexandrovska", Department Internal Medicine, Faculty of Medicine, Medical University – Sofia, Bulgaria

Abstract. *Obesity has been linked with vitamin D deficiency in a number of cross-sectional studies, reviews and meta-analyses. The aim of the present study was to assess the correlations of plasma 25(OH) vitamin D levels with indices of body composition examined by DXA with an emphasis on lean and bone mass as well as on indices such as android/gynoid fat, appendicular lean mass, fat-mass indexes (FMI) and fat-free mass indexes (FFMI). 62 adult subjects consented to participate – 27 men (43.5%) and 35 women (56.5%). Their mean age was 45.3 ± 9.5 years. Fan-beam dual-energy X-ray (DXA) body composition analysis was performed on a Lunar Prodigy Pro bone densitometer with software version 12.30. Vitamin D was measured by electro-hemi-luminescent detection as 25(OH) D Total (ECLIA, Elecsys 2010 analyzer, Roche Diagnostics). Statistical analyses were done using the SPSS 23.0 statistical package. The serum 25(OH)D level was correlated significantly only to the whole body bone mineral content, the appendicular lean mass index (ALMI) and the ALM-to-BMI index, underlining a predominant role for lean and fat-free mass. Vitamin D showed a very weak correlation to % Body Fat and the Fat Mass Index (FMI) in men only. Moreover, the multiple regression equation including the associated parameters could explain only 7% of the variation in the serum 25(OH) D levels. Our conclusion was, that there are differences in the associations of the vitamin D levels with the different body composition indices, but these associations are generally very weak and therefore – negligible.*

Key words: *Vitamin D, Body composition, correlations*

Corresponding author: *M. G. Nikolova, Department of Hygiene, Medical Ecology and Nutrition, Faculty of Medicine, Medical University of Sofia, 1, Zdrave str., Sofia 1431, Bulgaria, e-mail: marianikolova@mail.bg*

INTRODUCTION

Overweight and obesity have become an epidemic in westernized societies. So has vitamin D insufficiency and deficiency. Obesity has been linked with vitamin D deficiency in a number of cross-sectional studies, reviews and meta-analyses [1- 4]. The negative correlations of serum vitamin D levels with different indices of obesity, such as body weight, BMI, WC and waist-to-hip ratio (WHR), were extensively studied [1-5]. In a national representative sample individuals with 25(OH)D < 25 nmol/l showed a significantly higher incidence of obesity compared to those with higher vitamin D levels (57.8% vs. 42.2%, $p < 0.02$) [2]. Other studies have questionned the relationship between vitamin

D deficiency and obesity, highlighting the fact that no exact causal link had been proven yet [6]. Vitamin D is stored in the liver and adipose tissue [7]. Therefore it would be interesting to know which type of body tissue (lean, fat or bone) correlates better with the serum levels of 25(OH) vitamin D.

There are different techniques for body composition measurements in the clinical setting, such as computed tomography (CT), magnetic resonance imaging (MRI), dual X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). A few studies assessed the correlations of vitamin D levels with the subcutaneous and visceral fat combining data from CT and DXA [8-11]. In those studies plasma 25(OH) D concentrations were negatively associated with percent body fat and total adipose tissue. In the majority of them visceral fat (VAT) was better correlated to serum vitamin D than subcutaneous fat (SAT). On the other hand, the contribution of lean mass has been questioned in a few studies based on dual X-ray absorptiometry (DXA) and was generally found to be less important than that of fat mass [8,10]. The results were rather inconclusive showing association with the appendicular lean mass in one of those studies [8] and no association with lean mass in another one [10]. Data on the correlations of serum vitamin D with the body compartments coming from bioelectrical impedance analysis (BIA) are also focused on the fat mass (FM) and visceral fat mass (VFM) mainly, not on the fat-free mass (FFM) [12-17]. Most of them reported a positive association of plasma 25(OH)D with the total amount and percentage of body fat. So the question which body compartment – lean or fat, relates better to plasma 25(OH)D remains open. Moreover, the concept of age-related sarcopenia has brought into life a number of new indexes describing the shift towards fatness even in the presence of normal weight [18-22].

The aim of the present study was to assess the correlations of plasma 25(OH) vitamin D levels with indices of body composition examined by DXA with an emphasis on lean and bone mass as well as on derived indices such as android/gynoid fat, appendicular lean mass, fat-mass indexes (FMI) and fat-free mass indexes (FFMI). Our hypothesis was that plasma vitamin D would be better correlated to fat mass than to lean mass.

MATERIAL AND METHODS

Design

This is a cross-sectional observational study. It was approved by the responsible authorities at the Medical University and was in compliance with ethical standards and the Declaration of Helsinki. Each participant signed informed consent prior to any procedure. The inclusion criteria were age between 18 and 60 years and willingness to participate. The age range was selected to avoid the additional confounding influence on body composition of age-related sarcopenia. The exclusion criteria were severe or chronic diseases or medications known to affect body weight, immobilization, and others known to induce morbid obesity. Among the exclusion criteria were conditions such as heart failure NYHA III and IV, respiratory failure, chronic kidney disease stage III to V, liver cirrhosis, pancreatitis, musculoskeletal disorders (severe fractures, disability) etc. Among the medications that were not allowed were glucocorticoids, immunosuppressive drugs, antipsychotic drugs and others.

Subjects

The participants came from the general population. They were referred by their GPs for diet counseling in the setting of healthy lifestyle or to induce weight loss in those with overweight or obesity. 500 subjects were offered to participate in this study and 62 consented – 27 men (43.5%) and 35 women (56.5%). Their mean age was 45.3 ± 9.5 years. Their age distribution was as follows: 20-29 years – 2 men and 2 women; 30-39 years – 6 and 7 respectively, 40-49 years – 14 and 10; 50-59 years – 5 and 16.

Methods

Medical history was collected and anthropometric measurements were performed. Body weight was measured by a calibrated digital scale (Tanita BC 420 MA, Tanita Inc., Japan) to the nearest 0.1 kg in light clothes without shoes. Up to 1.0 kg was subtracted from the measured weight as remaining clothes. Body height was recorded in the upright position without shoes to the nearest 0.5 cm. BMI categories were calculated in kg/m^2.

The body composition analysis was performed in the early morning after an overnight fasting for at least 12 hours. The subjects were required to adhere to standard body composition testing guidelines, wearing light clothes [19, 23]. They were positioned lying supine with the entire body, including all soft tissue, within the table margins. The arms were positioned palm down with a space straight at the patient's sides; the legs were kept together with the feet relaxed. Fan-beam dual-energy X-ray (DXA) body composition analysis was performed on a Lunar Prodigy Pro bone densitometer (GE Lunar, Chicago, IL, USA). All DXA scans were read by the same technologist in a semi-automatic way including manual modifications of the regions of interest; software version 12.30. Body composition data were presented by the software as

FM in grams, LM in grams, and bone mineral content (BMC) in grams. The percentage of FM (% FM) was also calculated. Data were calculated separately for the different body sub-regions (arms, legs and trunk; android and gynoid), as well as total values according to the ISCD 2013 guideline [23]. Additionally a number of ratios were calculated – fat mass ratios (Trunk/Total, Legs/Total, Arms + Legs/Trunk, Android/Gynoid), as well as appendicular lean and fat mass (ALM and AFM, in kg). Height corrected parameters were calculated according to recent publications – fat mass index (FMI, in kg/m^2), fat-free mass index (FFMI, in kg/m^2), ALM index (ALM/height^2, in kg/m^2) and LM/Height^2 [18-21]. These indexes try to correct the confounding influence of body height and size on simple indices such as WC and FM (in kg).

The following reference ranges were used for the ALM and related fat-free (FFM) and lean body mass (LBM) indexes:

- *ALM*: for men 23.7-30.9 kg (< 23.7 = sarcopenia); for women 14.0-21.4 kg (< 14.0 = sarcopenia)
- *ALM index* = ALM/Height^2: for men 7.5-9.7 kg/m^2 (< 7.5 = sarcopenia); for women 6.4-8.2 kg/m^2 (< 6.4 = sarcopenia)
- *ALM-to-BMI ratio:* reference range for men above 1.109 (below = sarcopenia); for women above 0.734
- *Fat-free mass index*, *FFMI* = FFM (kg)/Height (m^2): reference range for men 21.8-24.4 kg/m^2; and for women – 17.1-18.4 kg/m^2.
- *Lean body mass index*, *LBMI = LBM/Height (m^2)*: reference range for men 15.9-19.9 kg/m^2; and for women – 13.1-16.3 kg/m^2.

The following reference ranges were used for the Fat mass index, FMI = FM(kg)/Height(m^2): for men the reference range was 3.0-6.0 kg/m^2 if BMI was normal; it was 6.0 to 9.0 kg/m^2 in overweight; 9.0-12.0 kg/m^2 in grade I obesity, 12.0 to 15.0 kg/m^2 in grade II obesity and above 15.0 kg/m^2 in grade III obesity. In women the respective BMI-adjusted reference ranges were 5.0-9.0 kg/m^2 (if BMI was normal), 9.0-13.0 kg/m^2 (overweight), 13.0-17.0 kg/m^2 (grade I obesity), 17.0-21.0 kg/m^2 (grade II obesity) and above 21.0 kg/m^2 (grade III obesity).

Blood samples were collected between 7:00 a.m. and 10:00 a.m. Plasma 25(OH) Vitamin D was measured by electro-hemi-luminescent detection as 25(OH)D Total (ECLIA on an Elecsys 2010 analyzer, Roche Diagnostics, Switzerland). The intra-assay error is 1.7-7.8%.

Statistical analysis

Statistical analyses were done using the SPSS 23.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA).Descriptive statistics and variation analysis were first performed. Inter-group comparisons were made via the Mann-Whitney and Cruscal-Wallis tests. Data were analyzed according to sex and BMI categories. ANOVA, correlation, simple and multiple linear regression analyses were performed. Obesity grade II and III were merged to increase the number of participants in this subgroup. Statistical significance was set as $p \leq 0.05$.

RESULTS

Serum levels of 25(OH) D of the whole group were 33.0 ± 17.3 nmol/l (median – 29.1 nmol/l). In men they were 36.5 ± 18.1 nmol/l; and in women 30.3 ± 16.3 nmol/l. The difference between both sexes was not significant. 48.4% of the study population had vitamin D insufficiency, 37.1% had deficiency, and only 3.2% had values above 30 ng/dl (75 nmol/l).

The descriptive statistics of the DXA-derived body composition data is presented in Table 1. As expected, men and women showed statistically significant differences in all parameters of body anthropometry and composition, except for age, BMI and Total body fat (in kg). Women had significantly higher values for % total body and regional fat (gynoid and arms), while men showed higher values of all remaining parameters.

Table 2 summarizes the data for the different indices describing fat-free and lean mass (LBMI, FFMI, ALM, ALMI and ALM-to-BMI ratio), as well as indices for fatness and obesity (Appendicular FM, FMI, A/G fat ratio, Trunk fat/Total fat ratio, Legs fat/Total fat ratio and Arms + Legs fat/Trunk fat ratio). Men showed significantly higher FFM, as well as higher Trunk/Total fat ratio, A/G fat Ratio, ALM, ALMI, ALM-to-BMI ratio, FFMI and LBMI. Women had higher values for the following fat mass ratios: Legs/Total fat, Arms + Legs/Trunk fat, FMI and appendicular fat mass. These results reflected the accumulation of more tissue in the abdominal region, as well as a tendency for higher muscle mass in men.

Approximately ¾ of the participants (75.8 %) had elevated A/G fat ratios. 79 % had ALM in the reference range, while 21% had elevated values. The ALMI was normal in 79%, elevated in 12.9% and subnormal – in 8.1%. Only 1 participant had normal ALM-to-BMI ratio, all others had suboptimal values. 51.6% had normal FFMI, the remaining ones had subnormal values. 74.2% had normal FMI, while 11.3% had increased values and 14.5% – decreased FMI. The LBMI was normal in 59.7% of the participants, and abnormally elevated – in 40.3%.

Table 3 shows the Spearman linear correlation coefficients describing the relationship between serum vitamin D levels and different body composition parameters and indices. Serum 25(OH) D correlated linearly with three parameters only: Total BMC, ALMI and ALM-to-BMI ratio. The correlation with the bone mineral content is logical and moderate, while the other two correlations with the appendicular muscle indices are weak. In men serum 25(OH) D were moderately and inversely correlated with Total % fat and FMI. The best regression curves for the whole group are shown in Figure 1.

In the multiple regression analysis ALM-to-BMI ratio showed the highest predictive power followed by the Total BMC. The final model in the backward procedure attained p = 0.024) and adjusted R2 = 0.067. The increase of ALM-to-BMI ratio with one point leads to an increase of serum vitamin D by 29 nmol/l. This regression model explained only 7% in the serum vitamin D variations. Similar results were obtained in men, while in women regression analyses were not performed because of lack of significance from the correlation analyses.

Table 1. The descriptive statistics of the whole-body and regional DXA body composition analysis is shown as means and SDs. Median values are shown for the group as a whole

Variable	Total group (N = 62)			Men (N = 27)		Women (N = 32)		P for the inter-group difference
	Mean	SD	Median	Mean	SD	Mean	SD	p
Age (years)	45.29	46.65	9.49	42.97	9.03	47.08	9.58	0.062
Weight (kg)	98.07	94.40	18.93	109.99	16.72	88.87	15.14	< 0.001
Height (cm)	170.11	168.00	9.67	178.15	6.96	163.91	6.30	< 0.001
BMI (kg/m2)	33.71	33.07	4.74	34.60	4.54	33.02	4.84	0.162
Total body lean mass, LM (kg)	56.23	55.12	12.92	68.62	8.21	46.67	5.67	< 0.001
Total Body % Fat, % TBF	44.04	6.86	43.60	38.38	4.75	48.41	4.71	< 0.001
Whole body fat mass, FM (kg)	41.31	9.58	41.48	41.37	10.20	41.27	9.22	0.967
Whole body bone mineral content, BMC (kg)	2.95	0.53	2.79	3.38	0.47	2.62	0.28	< 0.001
Gynoid % Fat	47.41	8.19	49.30	39.80	5.54	53.27	3.93	< 0.001
Gynoid Total Mass (kg)	14.49	2.74	13.87	15.64	2.81	13.61	2.37	0.003
Gynoid FM (kg)	6.78	1.59	6.79	6.31	1.71	7.15	1.40	0.039
Gynoid LM (kg)	7.62	1.89	7.41	9.33	1.43	6.31	0.87	< 0.001
Gynoid BMC (kg)	0.31	0.07	0.29	0.36	0.07	0.27	0.05	< 0.001
Gynoid Total Mass (kg)	14.80	2.78	14.15	16.00	2.83	13.88	2.39	0.002
Android % Fat	51.28	5.75	51.15	48.78	4.39	53.21	5.98	0.002
Android Total Mass (kg)	7.55	1.91	7.21	8.57	1.72	6.79	1.70	< 0.001
Android FM (kg)	3.96	1.25	3.72	4.32	1.18	3.68	1.26	0.013
Android LM (kg)	3.64	0.89	3.39	4.35	0.75	3.11	0.55	< 0.001
Android BMC (kg)	0.05	0.01	0.05	0.06	0.01	0.05	0.01	0.027
Trunk % Fat	46.42	5.28	46.550	43.39	4.10	48.76	4.92	< 0.001
Trunk Total Mass (kg)	47.46	10.55	44.870	53.10	9.03	43.43	9.77	< 0.001
Trunk FM (kg)	22.37	6.11	21.222	24.19	6.17	20.93	5.74	0.025
Trunk LM (kg)	25.20	5.58	25.020	29.78	4.17	21.92	3.90	< 0.001
Trunk BMC (kg)	0.92	0.24	0.893	1.08	0.20	0.80	0.19	< 0.001
Legs % Fat	44.51	10.09	44.650	35.30	6.53	51.61	5.55	< 0.001
Legs Total Mass (kg)	32.71	6.06	31.550	35.63	5.94	30.46	5.18	0.001
Legs FM (kg)	13.90	3.79	14.196	12.50	3.94	15.01	3.32	0.009
Legs LM (kg)	17.45	4.43	16.818	21.80	2.59	14.10	1.86	< 0.001
Legs BMC (kg)	1.12	0.22	1.110	1.31	0.16	0.99	0.15	< 0.001
Arms % Fat	41.11	10.09	40.300	32.11	6.26	48.06	6.22	< 0.001
Arms Total Mass (kg)	10.09	1.99	9.650	11.41	1.78	9.07	1.50	< 0.001
Arms FM (kg)	3.93	1.02	3.842	3.50	0.77	4.26	1.08	0.003
Arms LM (kg)	5.81	1.82	5.341	7.48	1.42	4.52	0.67	< 0.001
Arms BMC (kg)	0.35	0.11	0.328	0.42	0.10	0.29	0.06	< 0.001

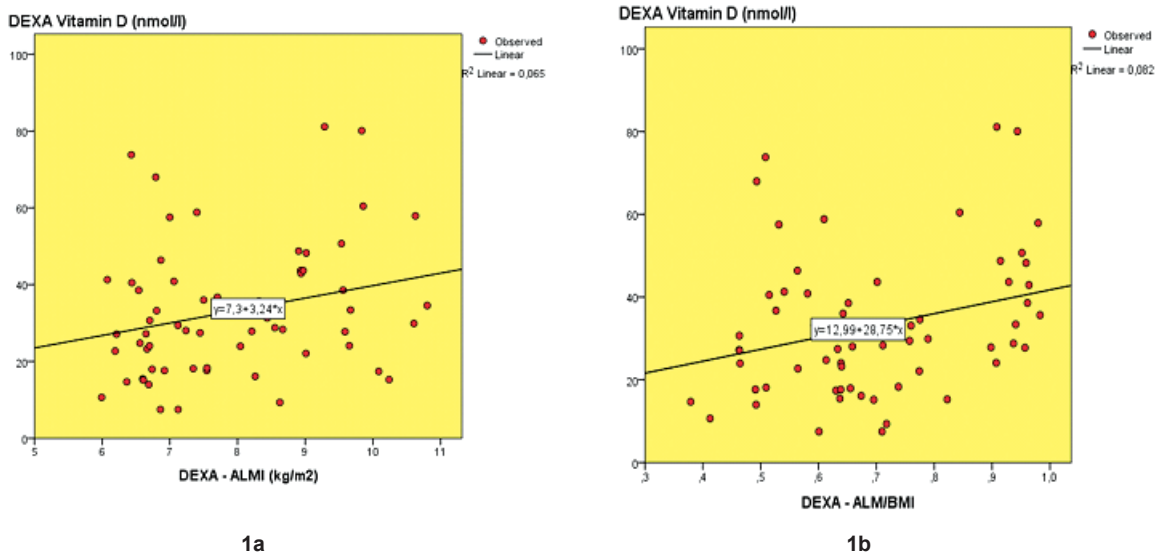
Table 2. The descriptive statistics of the DXA-derived body composition indices is presented as means and SDs. Median values are shown for the group as a whole

Variable	Total group (N = 62)			Men (N = 27)		Women (N = 32)		P for the inter-group difference
	Mean	Median	SD	Mean	SD	Mean	SD	p
LBMI = LBM/Height (kg/m^2)	18.18	18.37	2.73	20.53	2.08	16.37	1.51	< 0.001
FFMI = FFM/Height (kg/m^2)	19.19	19.36	2.76	21.60	2.05	17.34	1.53	< 0.001
Appendicular lean mass, ALM (kg)	23.26	21.43	5.91	29.28	2.88	18.61	2.30	< 0.001
ALMI = ALM/height2 (kg/m^2)	7.92	7.52	1.35	9.23	0.83	6.91	0.59	< 0.001
ALM-to-BMI ratio	0.70	0.66	0.17	0.86	0.11	0.57	0.09	< 0.001
Appendicular FM (kg)	17.78	18.63	4.47	16.00	4.38	19.20	4.08	0.005
FMI = FM/Height (kg/m^2)	14.52	14.30	3.67	13.00	3.04	15.69	3.71	0.003
Android/gynoid fat, A/G ratio	1.11	1.11	0.15	1.24	0.11	1.01	0.10	< 0.001
Trunk fat/Total fat ratio	0.54	0.54	0.06	0.58	0.04	0.50	0.05	< 0.001
Legs fat/Total fat ratio	0.34	0.34	0.05	0.30	0.04	0.37	0.04	< 0.001
Arms+Legs fat/Trunk fat ratio	0.81	0.81	0.18	0.67	0.11	0.92	0.15	< 0.001

Table 3. Correlation coefficients of serum vitamin D (as independent variable) and indices of anthropometry and body composition (as dependent variables) from the linear regression analysis

Dependent variables	Витамин D		
	Whole group	Men	Women
Age (years)	0.118	0.152	-0.004
Weight (kg)	-0.050	-0.219	-0.061
Height (cm)	0.087	0.106	-0.216
BMI (kg/m^2)	-0.095	-0.332	0.030
Trunk/total fat mass ratio	0.064	-0.132	-0.026
Legs/total fat mass ratio	-0.149	-0.026	-0.091
Arms+legs/Trunk fat mass ratio	-0.033	0.126	0.038
A/G Fat Ratio	0.227	0.046	0.085
Total body % fat	-0.230	-0.418*	-0.071
Total body Fat mass (kg)	-0.177	-0.364	-0.028
Total body Lean mass (kg)	0.114	-0.078	-0.142
Total body BMC (kg)	0.307*	0.496**	0.072
Gynoid Total Tissue (kg)	-0.121	-0.291	-0.111
Gynoid Fat mass (kg)	-0.216	-0.330	-0.077
Gynoid Lean mass (kg)	0.110	-0.120	-0.103
Gynoid BMC (kg)	0.175	0.194	0.134
Android Total Tissue (kg)	-0.081	-0.192	-0.036
Android Fat mass (kg)	-0.115	-0.341	-0.040
Android Lean mass (kg)	0.071	-0.145	-0.129
Android BMC (kg)	0.175	0.358	0.111
Trunk Total Tissue (kg)	0.019	-0.135	-0.030
Trunk Fat mass (kg)	-0.095	-0.374	0.010
Trunk Lean mass (kg)	0.004	0.021	-0.174
Trunk BMC (kg)	0.213	0.251	0.020
ALM (kg)	0.231	0.323	-0.014
ALMI (kg/m^2)	0.278*	0.256	0.157
ALM-to-BMI ratio	0.298*	0.631***	-0.011
FMI (kg/m^2)	-0.230	-0.455*	-0.030
FFMI (kg/m^2)	0.147	-0.110	0.033
LBMI (kg/m^2)	0.134	-0.144	0.027

* if $p < 0.05$, ** if $p < 0.01$, *** if $p < 0.001$



1a 1b

Fig. 1. The significant albeit very weak correlations of serum vitamin D with the ALMI (1a) and ALM/BMI (1b) are shown below

DISCUSSION

In this study we tested the hypothesis, whether there are differences in the associations of serum vitamin D levels with the different body composition compartments (fat and lean mass). Our hypothesis was that fat mass would be better correlated to the 25(OH)D level. Surprisingly, it was correlated significantly only to the whole body bone mineral content, the appendicular lean mass index (ALMI) and the ALM-to-BMI index, underlining a predominant role for lean and fat-free mass. Vitamin D showed a very weak correlation to % Body Fat and the Fat Mass Index (FMI) in men only. Moreover, the multiple regression model including the associated parameters could explain only 7% of the variation in the serum 25(OH)D levels. Our conclusion was, that there are differences in the associations of the vitamin D levels with the different body composition indices, but these associations are generally very weak and therefore – negligible. As a collateral finding, higher fat-free mass (FFM) was found in men, with visceral obesity (increased android-to-gynoid ratio) being highly prevalent in this study population.

Our initial hypothesis was based mainly on data coming from body impedance analyses, showing a better correlation of serum vitamin D with FM, rather than with FFM [12, 15]. In the study by Vilarrasa et al. 25(OH) D was stronger correlated with body fat (r = -0.53) and fat mass (r = -0.44), than with fat-free mass (r = -0.35) [12]. In the study by Jungert et al. 25(OH) D was associated with total body fat in women, but not in men [15]. No correlation with FFM was found in this study. Data coming from studies using the DXA technology for body composition analysis are even scarcer. They are mainly focused on visceral and subcutaneous fat [11]. In the study by Lenders et al. the correlation coefficient of serum vitamin D versus FM was r = -0.3 ($p < 0.05$), while it was -0.16 with FFM and not significant ($p > 0.05$) [10]. Seo et al. reported negative correlations of serum 25(OH) D with body fat percentage, but positive ones with appendicular skeletal muscle mass [8]. A number of DXA-based studies focused on ALM and different indices of body fatness or muscle mass, but they were designed to assess the prevalence and characteristics of sarcopenia, not to explore associations with vitamin D status [20, 22].

Having in mind the scarcity of the data in the literature we think that the associations of vitamin D with lean mass and related indices are promising for further research in that area. However, our study has a number of limitations. The results are not representative for the general population. The study population is of moderate size and significance is lost if subgroups are stratified according to BMI, age etc. On the other hand, this is one of the few studies using DXA and indices of body fatness and muscle mass in assessing their relation to serum levels of 25(OH) D in adult people throughout the whole continuum of normal weight, overweight and obesity. It underlines the role of vitamin D as a pro-hormone being correlated with body composition.

In conclusion, we were able to prove that serum 25(OH) D is weakly correlated to BMC, ALMI and ALM-to-BMI, and not to % BF or FM. The contribution of the vitamin D status to the body composition is negligible. This information could be useful in combined studies of vitamin D deficiency and obesity.

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