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Biomarkers of vitamin D status in healthy adults: associations with serum lipid parameters – a pilot study

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Abstract

Vitamin D deficiency is among important healthcare challenges today. Traditionally, vitamin D status is assessed through determination of 25-hydroxy metabolite (25(OH)D), but novel data point to 24,25$(OH)_2$D and 25(OH)D/24,25$(OH)_2$D ratio (VDMR) as promising biomarkers. It is widely accepted that the biological role of vitamin D exceeds its well-known contribution to bone turnover. However, its effects on overall energy metabolism and lipid status alterations are not completely understood. In this study, we analyzed the relationship of vitamin D status assessed as concentrations of 25(OH)D3 and 24,25$(OH)_2$D3 determined by liquid chromatography-tandem mass spectrometry, as well as VDMR with advanced lipid status parameters. Vitamin D status biomarkers, routine parameters of lipid status and size and distribution of lipoprotein subclasses were determined in 89 healthy adults (35 with adequate vitamin D status and 54 with vitamin D deficiency). Our results indicated a preponderance of pro-atherogenic small, dense LDL particles (sdLDL) in vitamin D deficient subjects. Both 25(OH)D and 24,25$(OH)_2$D were associated with a relative proportion of sdLDL (B: -0.410; SE: 0.154; P=0.010; and B: -2.041; SE: 0.969; P=0.039, respectively). Positive correlation was found for VDMR and relative proportion of HDL 3a particles (ρ=0.251; P=0.024). VDMR value was decreased in subjects with vitamin D deficiency (P=0.001), thus implying its usefulness as a biomarker. A thorough investigation of novel vitamin D biomarkers and advanced lipid status parameters can be useful in the estimation of individual risk for the development of cardiometabolic alterations.

Keywords: vitamin D biomarkers, 25(OH)D3; 24,25$(OH)_2$D3, VDMR, sdLDL, advanced lipid status

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Introduction

Global data point to a pandemic of vitamin D deficiency in the modern world (1,2). This secosteroid is historically considered to be a vitamin involved in the control of calcium and phosphate homeostasis. However, modern understanding of vitamin D is far more extensive, and it is widely accepted that it is a hormone with multiple complex roles that are still not fully understood (3,4). Given the fact that vitamin D deficit is repeatedly observed in various systemic diseases, investigations aiming to elucidate these associations are highly needed.

One of the intriguing functions of vitamin D is related to its contribution to overall changes of energy metabolism. Namely, it is well-known that obesity is coherent with vitamin D deficiency, but specific mechanisms of such an association are not clear (5). Since dyslipidemia is among the principal features of obesity, many previous studies have analyzed the relationship and possible molecular mechanism that could be responsible for the presumable role of vitamin D deficiency in the development of lipid status alterations and vice versa. However, consistent conclusions have not been obtained so far. It has been shown that vitamin D is in correlation with high-density lipoprotein cholesterol (HDL-C) levels, but prior studies have not yielded univocal conclusions regarding the associations of vitamin D and total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels (6). It should also be noted that previous investigations were largely focused on routine lipid status parameters, while the relation between vitamin D and markers of advanced lipid status, such as size and relative proportion of lipoprotein subclasses, has scarcely been explored so far. Yet, having in mind that the modern scientific approach to dyslipidemia pays significant attention to the quality of lipoprotein species and distribution of their subclasses (7), the plausible association of vitamin D with specific lipoprotein subfractions should be evaluated.

Vitamin D status is traditionally assessed through laboratory determination of 25-hydroxy vitamin D (25(OH)D) concentration in plasma, since this is the most abundant and stable vitamin D precursor. However, novel research points towards other vitamin D metabolites that could function as more accurate biomarkers of its status in the body. In this regard, 24,25-hydroxy vitamin D ($24,25(OH)_2D$) has been revealed as a highly promising parameter (8). $24,5(OH)_2D$ is a degradation product of vitamin D metabolism, but it can compete with the bioactive form for vitamin D receptor. Therefore, the ratio $25(OH)D/24,25(OH)_2D$, known as vitamin D metabolite ratio (VDMR) could be used as a biomarker of a balance between active and non-active fraction of vitamin D (9). Yet, it should be noted that the association between new vitamin D status markers and lipid status parameters has not been analyzed so far.

The aim of this study was to analyze the relationship of traditional and novel biomarkers of vitamin D with routine and advanced lipid status parameters in a group of healthy, middle-aged subjects. We also sought to explore whether any of the analyzed indicators of vitamin D status were independently associated with alterations of lipid profile.

Subjects and methods

Subjects

Eighty-nine healthy adult volunteers (39 women and 50 men) of Caucasian origin were enrolled in this study. The participants were recruited during regular annual check-ups in healthcare centers. The exclusion criteria were as follows: minor age, presence of any acute or chronic illness, use of any hypolipemics and administration of vitamin D supplements and/or calcium. All subjects provided the necessary anthropometric data (age, gender, weight, height and lifestyle habits) by fulfilling a standardized questionnaire. Body-mass index (BMI) was determined as weight/(height)^2.

The entire study protocol was designed and conducted according to the ethical guidelines defined by the Helsinki Declaration. All subjects signed an informed consent prior to the enrollment.

Sampling and laboratory methods

Blood samples were drawn into blood collection tubes after overnight fasting. Plasma and serum samples were separated and stored at -80^{0}C until the analyses. Routine biochemical parameters were determined by standard laboratory methods applied on an automated analyzer Ilab 300 Plus (Instrumentation Laboratory, Milan, Italy). Lipoprotein subclasses were separated by the method of polyacrylamide gradient gel (3-31%) electrophoresis according to the previously published procedure (10) applied on the vertical electrophoresis system Hoefer SE 600 Ruby and (Amersham Pharmacia Biotech, Vienna, Austria). The determination of dominant lipoprotein particle size was performed by using the Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria), Magic Scan software (version 4.6;1999; UMAX Data Systems, Inc) and Image Quant software (version 5.2;1999; Molecular Dynamics).

Vitamin D metabolites (25(OH)D3 and 24,25(OH)$_2$D3) were separated and quantified by the liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Agilent, Santa Clara, USA), by using the in-house developed procedure (11). VDMR for each subject was calculated by dividing 25(OH)D3 and 24,25(OH)$_2$D3 levels. Vitamin D deficiency was defined according to the guidelines of the European Endocrine Society and the Endocrine Society of USA (12).

Statistical methods

The Shapiro-Wilk test was used for testing the distribution of the analyzed data. Normally distributed variables were presented as mean ± standard deviation and compared using the Student t-test, whilst asymmetrically distributed data were presented as median (interquartile range) and compared by the Mann-Whitney U-test. Categorical data were presented as absolute frequencies and compared by the Chi-square test. Spearman correlation analysis and general linear regression were used to search for independent associations between vitamin D status and other examined markers.

Results

General characteristics of the study population are presented in Table I. Approximately 60% of study participants were overweight and approximately 15% of them were obese. Since sampling was performed during summer and winter season, we compared the levels of vitamin D parameters among subgroups of examinees clustered according to the sampling period (June-September: 26 samples, November-April: 63 samples). The obtained data revealed significantly higher concentrations of 25(OH)D3 (median (IQR): 22,16 (16,24-31,43) ng/mL *vs* 17,84 (13,54-20,63) ng/mL; P = 0.003) and 24,25$(OH)_2$D3 (median (IQR): 3,54 (3,01-5,48) ng/mL *vs* 3,10 (2,63-3,72) ng/mL; P = 0.004) in samples taken during summer. In contrast, values of VDMR were comparable among these groups (P = 0.796). We found no differences among men and women in 25(OH)D3 (median (IQR): 19.68 (15.80-23.16) vs 18.20 (15.37-27.88); P=0.556), 24,25(OH)2D3 (median (IQR): 3.25 (2.67-4.20) vs 3.35 (2.74-3.86); P=0.862) and VDMR (median (IQR): 5.61 (5.14-6.98) vs 5.44 (4.72-6.39); P=0.108)

Table I General characteristics of the study population
Tabela I Opšte karakteristike ispitivane populacije

Parameter	
Age (years)	54.4 ± 7.57
Gender (m/f)	50/39
BMI (kg/m^2)	26.3 ± 3.67
Smoking (yes/no)	25/61
Physical activity (yes/no)	70/19
Alcohol intake (yes/no)	46/43

Data are presented as mean ± standard deviation for continuous variables and as absolute frequencies for categorical variables

Table II represents the differences in anthropometric and biochemical characteristics between study participants with and without vitamin D deficiency, defined as 25(OH)D3 level lower or equal and/or higher than 20 ng/mL. The only statistical significance was found for glucose concentration, which was higher in vitamin D deficient subjects. In addition, we observed markedly higher BMI and lower corrected calcium concentrations in vitamin D deficient examinees, although statistical significance was not reached (Table II).

Table II Anthropometric and biochemical parameters in study participants with adequate vitamin D status and vitamin D deficiency

Tabela II Antropometrijski i biohemijski parametri kod ispitanika sa adekvatnim nivoom i sa deficijencijom vitamina D

Parameter	Adequate vitamin D status (N = 35)	Vitamin D deficiency (N = 54)	P
Age (years)	53.7 ± 7.16	54.9 ± 7.56	0.439
Gender (m/f)	19/16	31/23	0.829
BMI (kg/m^2)	25.5 ± 3.19	26.9 ± 3.91	0.095
Total protein (g/L)	72.1 ± 5.39	73.9 ± 7.37	0.217
Albumin (g/L)	46.5 ± 3.25	47.6 ± 4.43	0.209
Glucose (mmol/L)	5.2 ± 0.74	5.7 ± 1.04	0.030
TC (mmol/L)	5.7 ± 1.05	5.6 ± 0.99	0.653
LDL-C (mmol/L)	3.8 ± 0.94	3.6 ± 0.92	0.221
HDL-C (mmol/L)	1.2 ± 0.44	1.4 ± 0.52	0.203
TG (mmol/L)	1.4 ± 0.45	1.4 ± 0.48	0.785
Urea (mmol/L)	4.7 ± 1.36	5.0 ± 1.26	0.378
Creatinine (μmol/L)	82.8 ± 12.49	81.0 ± 12.64	0.676
Uric acid (μmol/L)*	260.38 (235.57-309.58)	281.38 (231.57-328.83)	0.460
Calcium (mmol/L)*	2.39 (2.28-2.50)	2.27 (2.16-2.43)	0.059
Phosphate (mmol/L)*	1.01 (0.79-1.19)	1.06 (0.89-1.38)	0.729

Data are presented as mean ± standard deviation and compared by the Student-t test.

*Data are presented as median (IQR) and compared by the Mann-Whitney U-test.

The analysis of vitamin D status parameters revealed that the concentrations of 25(OH)D3, 24,25(OH)$_2$D3 and VDMR were lower in subjects with vitamin D deficiency (Table III).

Table III Vitamin D biomarkers in study participants with adequate vitamin D status and vitamin D deficiency

Tabela III Biomarkeri vitamina D kod ispitanika sa adekvatnim statusom i sa deficijencijom vitamina D

Parameter	Adequate vitamin D status (N = 35)	Vitamin D deficiency (N = 54)	P
25(OH)D3 (ng/mL)	27.52 (22.54-32.01)	15.99 (13.63-18.20)	< 0.001
24,25(OH)$_2$D3 (ng/mL)	4.24 (3.28-5.38)	2.79 (2.44-3.29)	< 0.001
VDMR	6.24 (5.33-7.24)	5.26 (4.73-5.88)	0.001

Data are presented as median (IQR) and compared by the Mann-Whitney U-test.

Next, we explored the variations in advanced lipid status parameters between the two analyzed groups (Table IV). However, we found no differences in the examined parameters, although a shift of LDL and HDL subclasses distribution towards smaller, more atherogenic particles was noticed.

Table IV Particle size and distribution of LDL and HDL subclasses in study participants with adequate vitamin D status and vitamin D deficiency

Tabela IV Veličina i raspodela LDL i HDL subfrakcija kod ispitanika sa adekvatnim nivoom i sa deficijencijom vitamina D

Parameter	Adequate vitamin D (N = 35)	Vitamin D deficiency (N = 54)	P
LDL particle size (nm)	26.5 ± 1.24	26.4 ± 1.50	0.918
LDL I (%)	24.2 ± 9.88	23.1 ± 9.94	0.604
LDL IIA (%)	13.0 ± 3.73	11.8 ± 3.89	0.150
LDL IIB (%)	14.9 ± 3.75	13.9 ± 2.72	0.156
LDL II (%)	27.9 ± 6.09	25.7 ± 5.46	0.081
LDL IIIA (%)	13.5 ± 3.35	14.1 ± 3.96	0.480
LDL IIIB (%)	7.0 ± 2.15	7.6 ± 2.19	0.169
LDL III (%)	20.5 ± 4.77	21.7 ± 4.87	0.245
LDL IVA (%)	12.0 ± 4.27	12.6 ± 4.26	0.562
LDL IVB (%)	15.3 ± 5.57	16.9 ± 5.39	0.196
LDL IV (%)	27.3 ± 8.54	29.4 ± 8.74	0.271
sdLDL (%)	47.8 ± 12.56	51.2 ± 11.86	0.213
HDL particle size (nm)	9.9 ± 1.04	9.6 ± 0.98	0.233
HDL 2b	39.7 ± 6.37	38.6 ± 8.77	0.541
HDL 2a	22.9 ± 3.08	22.6 ± 3.90	0.671
HDL 3a	19.2 ± 3.54	19.2 ± 3.93	0.981
HDL 3b	9.4 ± 2.89	9.7 ± 3.37	0.742
HDL 3c	8.7 ± 3.30	9.9 ± 4.28	0.171
Small HDL subclasses (%)	37.4 ± 7.38	38.1 ± 11.09	0.754

Data are presented as mean ± standard deviation and compared by the Student-t test.

To further explore the observed trend of positive associations between vitamin D deficiency and alterations of lipid homeostasis, we divided study participants in groups according to quartile values of 25(OH)D concentrations. When we compared lipid status parameters between the subjects clustered in the 1st quartile and 4th quartile groups, we found statistically significant differences in the distribution of LDL subclasses. Namely, the percentages of LDL IIA and total LDL II subclasses were decreased, while relative

proportions of LDL III and sdLDL particles were higher in the examinees with lower 25(OH)D3 concentrations. In contrast, we found no differences in the distribution of HDL subclasses (Table V). Similarly, there were no differences in the levels of routine lipid status parameters among quartiles of 25(OH)D3 concentrations (data not shown).

Table V Particle size and distribution of LDL and HDL subclasses between study participants clustered into subgroups according to quartiles of 25(OH)D3 concentrations

Tabela V Veličina i raspodela LDL i HDL subfrakcija kod ispitanika razvrstanih u grupe prema kvartilima koncentracije 25(OH)D3

Parameter	25(OH)D3>23,07 ng/mL (N = 22)	25(OH)D3<14,44 ng/mL (N = 22)	P
LDL particle size (nm)	26.57 (25.58-27.34)	27.02 (25.75-27.53)	0.573
LDL I (%)	20.36 (18.04-32.07)	18.81 (16.52-27.95)	0.342
LDL IIA (%)	13.38 (11.13-16.60)	10.42 (9.40-12.95)	0.008
LDL IIB (%)	14.74 (12.40-17.72)	13.11 (12.12-14.73)	0.080
LDL II (%)	28.11 (24.26-33.18)	24.83 (22.63-25.89)	0.009
LDL IIIA (%)	13.44 (10.33-15.40)	14.33 (13.43-15.79)	0.113
LDL IIIB (%)	6.49 (4.92-8.13)	7.71 (5.40-9.30)	0.124
LDL III (%)	20.32 (15.05-22.43)	22.89 (20.97-24.42)	0.036
LDL IVA (%)	11.15 (8.27-13.87)	12.79 (9.87-15.44)	0.177
LDL IVB (%)	16.03 (11.58-18.97)	17.05 (13.09-20.40)	0.342
LDL IV (%)	28.30 (19.85-33.00)	31.32 (23.98-35.43)	0.149
sdLDL (%)	48.55 (36.92-54.22)	55.46 (47.17-60.94)	0.047
HDL particle size (nm)	10.36 (8.59-10.64)	9.99 (8.69-10.32)	0.250
HDL 2b	38.38 (35.29-44.10)	36.27 (32.23-39.43)	0.130
HDL 2a	22.79 (21.73-24.76)	23.06 (20.17-25.58)	0.769
HDL 3a	19.98 (17.21-22.30)	21.54 (16.99-22.91)	0.148
HDL 3b	9.04 (7.91-11.44)	9.86 (8.44-12.38)	0.474
HDL 3c	8.38 (6.25-10.72)	9.98 (6.06-14.20)	0.163
Small HDL subclasses (%)	37.96 (34.17-40.80)	41.26 (35.66-44.55)	0.113

Data are presented as median (IQR) and compared by the Mann-Whitney U-test.

In the next step, we examined the correlations between vitamin D status biomarkers and parameters of lipid status in the entire study group. Significant correlations are presented in Table VI. Both 25(OH)D3 and 24,25$(OH)_2$D3 were in positive correlation with larger and in negative correlation with smaller LDL subclasses. In addition, VDMR was positively associated with relative abundance of HDL 3a subclasses.

Table VI Significant correlations between vitamin D status biomarkers and parameters of advanced lipid profile

Tabela VI Značajne korelacije između biomarkera statusa vitamina D i parametara proširenog lipidnog profila

	LDL IIA (%)	LDL IIB (%)	LDL II (%)	LDL IIIB (%)	LDL III (%)	sdLDL (%)	HDL 3a (%)
25(OH)D3 (ng/mL)	0.280**	0.213*	0.281**		-0.219*	-0.225*	
$24,25(OH)_2D3$ ng/mL	0.322**		0.306**	-0.236*	-0.292**	-0.275*	
VDMR							0.251*

Data represent Spearman's correlation coefficient. * P<0.05; ** P<0.01

Finally, we searched for independent associations of 25(OH)D3 and $24,25(OH)_2D3$ with an increased presence of sdLDL particles. Linear regression analysis revealed that 25(OH)D3 is an independent inverse determinant of relative proportions of sdLDL particles (B: -0.410; SE: 0.154; P=0.010) in the model that contained traditional contributing factors to sdLDL abundance (age, male gender, BMI and TC and TG concentrations). Similarly, $24,25(OH)_2D3$ concentration was independently and inversely associated with the prevalence of sdLDL in the equivalent model (B: -2.041; SE: 0.969; P=0.039).

Discussion

The results of this study suggest the presence of an independent association between decreased biomarkers of vitamin D status and altered lipid profile in otherwise healthy adults. 25(OH)D is commonly used as a reliable biomarker of vitamin D status, but in this study, we used the analytical advantage provided by an in-house developed LC-MS/MS method, which allows simultaneous determination of 25(OH)D3 and $24,25(OH)_2D3$ and subsequent mathematical estimation of their ratio VDMR. In general, the LC-MS/MS method is capable of separating and determining both vitamin D3 and D2 metabolites, but in this study, we opted for an analysis of vitamin D3 metabolites solely, since vitamin D3 (cholecalciferol) is a dominant form that is endogenously synthetized, and our examinees did not use any vitamin D2 supplementation. Novel research frequently emphasizes the significance of $24,25(OH)_2D3$, as a reliable biomarker of vitamin D status (13,14); therefore, its determination alongside 25(OH)D3 measurement allows a more precise insight into complex vitamin D metabolome and its biological functions.

Considering that the most important determinant of vitamin D status is its dermal synthesis provoked by sunlight exposure, we compared the levels of analyzed vitamin D

metabolites in samples collected during summer and winter season. Expectedly, levels of 25(OH)D3 were higher in the samples collected during summer, but our results also revealed a significantly higher concentration of 24,25$(OH)_2$D3 in these samples. It has been shown that 24,25$(OH)_2$D3 is the dominant metabolite of 25(OH)D3 (12), so higher dermal synthesis of cholecalciferol and a higher rate of its metabolic transformation to 25(OH)D3 imply increased formation of 24,25$(OH)_2$D3 too. In line with this, VDMR remained unchanged among samples taken in various seasons, thereby confirming that the entire metabolic cascade of vitamin D hydroxylation is driven by the level of its precursors' dermal synthesis. It should be noted that similar studies conducted with a larger sample size demonstrated that the ratio of vitamin D metabolites may vary according to the season (15,16). The small sample size in our study might be responsible for such a discrepancy in the results. However, it should be noted that a study by Toribio et al. conducted in a large group of premenopausal women did find higher values of 24,25$(OH)_2$D3/25(OH)D3 during summer and fall, but in the same study, this ratio did not differ in subgroups divided according to weekly sun exposure (15). Moreover, in a study by Ginsberg et al., 24,25$(OH)_2$D3 and 25(OH)D3 levels were highest during the spring and summer, but quite oppositely, their ratio was higher during winter and fall season (16). Thus, the issue of seasonal impact on VDMR should be further explored.

The principal aim of our study was to explore the relation between vitamin D deficiency and alterations of serum lipid profile. However, it should be noted that the majority of our participants were overweight and obese. The available data for Serbia show that 57.1% of population is overweight and 20.8% is obese (17). Having in mind that vitamin D deficiency is frequent in obese subjects, such prevalence of obesity in our study group might reflect on the frequency of vitamin D deficit. Indeed, 54 out of 89 subjects had 25(OH)D3 levels lower than 20 ng/mL. Moreover, a higher BMI was recorded in our examinees with vitamin D deficiency, although with borderline significance (Table II).

The association between lack of vitamin D and obesity is well known, but the underlying mechanisms are not completely understood. Several hypotheses are proposed to explain such a relationship. First of all, overweight and obese persons are usually less physically active and spend less time in the open air; thus, the sunlight exposure is diminished and consequently less vitamin D is produced (5). The weak point of this hypothesis is that not all obese persons are physically inactive, yet vitamin D deficiency is still prevalent in these individuals. The second hypothesis relays on the characteristic distribution of vitamin D in obese people. Being liposoluble, vitamin D is sequestrated in adipose tissue and thereby its level in plasma is decreased (18). Finally, the hypothesis of "volumetric dilution" implies that increased body volume in obese subjects simply causes the dilution of their vitamin D content (19). A common feature of all three hypotheses is that vitamin D deficiency is observed as a consequence, rather than a possible cause or risk factor of obesity. However, novel investigations indicate a possible role of vitamin D in adipose tissue modulation. Vitamin D receptors are present in adipocytes as well, and it has been shown that autonomous bioactivation of vitamin D occurs in this tissue

(20). Based on the findings that vitamin D stimulates the differentiation of preadipocytes to mature insulin-sensitive adipocytes (21), as well as apoptosis (5), a hypothesis on the role of vitamin D in "healthy remodeling" of adipose tissue has been raised.

Our results did not show any significant differences in routine and advanced lipid status parameters between the subjects with and without vitamin D deficiency, although trends toward the diminishing of LDL and HDL particle size were observed (Tables II and IV). The association between vitamin D and lipid metabolism is still not adequately explained. Previous studies consistently demonstrated a correlation between lower vitamin D status and decreased HDL-C level, while the results regarding the relationship of TC, LDL-C and TG with vitamin D biomarkers are conflicting (6). In order to more precisely estimate the contribution of vitamin D deficiency to alterations of lipid profile, we compared advanced lipid status parameters between the subjects who belong to the 1^{st} and 4^{th} quartile of 25(OH)D3 levels (Table V). A statistically significant shift toward smaller LDL subclasses was recorded in individuals with the lowest 25(OH)D3 concentrations. SdLDL particles have prominent pro-atherogenic potential, and our findings suggest that a complete insight into changes of lipid metabolism in vitamin D deficient subjects is possible only when both quality and quantity of lipoprotein particles are taken into account.

The relationship between lipids and vitamin D metabolism should be analyzed from several perspectives. Most importantly, vitamin D and cholesterol share common biosynthetic pathways, since 7-dehydrocholesterol is a precursor for both compounds. In line with this, an increase of cholesterol synthesis is associated with a decrease of cholecalciferol formation and vice versa (22). Such an assumption can be used as an explanation for the beneficial effects of statins on vitamin D level (23). Moreover, it has been shown that the active form of vitamin D stimulates the activity of 7α-hydroxylase, which is the regulatory enzyme of bile salt synthesis, and therefore responsible for reducing cholesterol levels (26). Finally, indirect effects of vitamin D should not be neglected, since vitamin D reduces the synthesis and secretion of parathormone, and it has been demonstrated that hyperparathyroidism stimulates alterations of serum lipids (25).

Importantly, our results suggest that both 25(OH)D3 and 24,25$(OH)_2$D3 are independently associated with prevalence of sdLDL. Although we did not observe any significant changes in HDL particle distribution in subjects with vitamin D deficiency, it should be noted that VDMR was in positive correlation with the proportion of smaller HDL 3a particles (Table VI). The relatively small sample size is likely the cause for the absence of additional significant correlations, so these findings should be evaluated in larger studies. It should, however, be noted that both vitamin D metabolites were in correlation with summary parameters for LDL II and LDL III subclasses, as well as with the proportion of sdLDL, as the most important integrative indicator of atherogenic lipoprotein profile. Previous studies suggested a possible active role of vitamin D in the regulation of lipid homeostasis, since a bidirectional relation was observed between HDL-

C and 25(OH)D3 (20). Taken together, a low level of vitamin D might be not merely a reflection of an altered lipid metabolism, but also the reason for such changes.

Of note, our analysis of associations of several vitamin D biomarkers with serum lipid parameters has revealed similar patterns of associations for 25(OH)D3 and 24,25$(OH)_2$D3. 24,25$(OH)_2$D3 is frequently emphasized as a promising biomarker of total vitamin D status (8), especially in relation to 25(OH)D3 as VDMR. An interesting finding of our study is that, although both 25(OH)D3 and 24,25$(OH)_2$D3 were lower in individuals with vitamin D deficiency, their VDMR was also decreased (Table III), thus suggesting a shift in the entire vitamin D metabolism towards degradation. A study by Battachi and collaborators (26) has demonstrated changes in activation/deactivation processes within vitamin D metabolism after supplementation. These findings emphasize the complexity of vitamin D metabolism and versatility of their metabolites, so future studies in this area are warranted. Furthermore, since VDMR illustrates the relationship between activation and inactivation phases of vitamin D metabolism, it could be very useful as an accurate biomarker of vitamin D status.

Several important drawbacks should be mentioned. First of all, the small sample size is a major limitation that prevents us from drawing any definitive conclusions. However, in this pilot study we pointed towards potentially important trends in the associations of vitamin D and advanced lipid status biomarkers, which should be further explored in large-scale studies. The associations of vitamin D biomarkers with the size and distribution of lipoprotein subclasses have scarcely been investigated so far, so our preliminary findings might be helpful in directing future research. Furthermore, even though seasonal variations are probably the most important contributors to the alterations in vitamin D status, samples in this study were taken during the entire year. However, in this study, we were focused on the investigation of the specific relationship between vitamin D metabolites and lipid status biomarkers, regardless of the season. Moreover, our results demonstrated that VDMR was unchanged in different seasons, thus suggesting that, even if the season basically affects the availability of the precursor for active vitamin D synthesis, the metabolism of vitamin D is essentially the same and driven by the intrinsic mechanisms which are unaffected by seasonal variations. Furthermore, it should be noted that important confounders of both vitamin D and lipid status biomarkers should be included in the assessment of an independent relationship between these elements of individual metabolic profile. However, the small sample size did not allow us to perform extensive multivariate analysis at present. Further studies are needed to resolve this issue.

In summary, our study demonstrated a significant and independent association of vitamin D deficiency and pro-atherogenic changes of lipoproteins. These finding might provide additional evidence for the hypothesized contribution of low vitamin D levels to the development of cardiometabolic diseases. Moreover, novel vitamin D biomarkers, such as 24,25$(OH)_2$D3 and VDMR, might be useful for a comprehensive assessment of vitamin D status.

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Biomarkeri statusa vitamina D kod zdravih odraslih osoba: povezanost sa serumskim lipidnim parametrima – pilot studija

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Kratak sadržaj

Deficijencija vitamina D je jedan od značajnih izazova za zdravstvene sisteme današnjice. Tradicionalno, status vitamina D se procenjuje na osnovu određivanja koncentracije njegovog 25-hidroksi metabolita (25(OH)D), ali novi podaci ukazuju na potencijalni značaj $24,25(OH)_2D$ i odnosa $25(OH)D/24,25(OH)_2D$ (VDMR). Danas je široko prihvaćeno da biološka uloga vitamina D uveliko prevazilazi njegove efekte na koštani promet. Međutim, još uvek nije u potpunosti jasan uticaj vitamina D na energetski metabolizam i izmene lipidnog statusa. U ovom istraživanju ispitivali smo povezanost između statusa vitamina D, procenjenog na osnovu nivoa 25(OH)D3 i $24,25(OH)_2D3$ koji su određeni metodom tečne hromatografije – tandem masene spektrometrije, te VDMR sa standardnim i novim parametrima lipidnog statusa. Ispitivani analiti određeni su kod 89 zdravih odraslih osoba. Naši rezultati ukazuju na povećan udeo malih gustih LDL čestica (sdLDL) kod osoba sa deficijencijom vitamina D. Koncentracije 25(OH)D i $24,25(OH)_2D$ su bile nezavisno povezane sa relativnim udelom sdLDL (B: -0,410; SE: 0,154; P=0,010; i B: -2,041; SE: 0,969; P=0,039, redom). Uočena je pozitivna korelacija između VDMR i relativnog udela HDL 3a (ρ=0,251; P=0,024). VDMR je snižen kod osoba sa deficijencijom vitamina D (P=0,001), što implicira njegov značaj kao potencijalnog biomarkera. Detaljno ispitivanje novih biomarkera statusa vitamina D i parametara lipidnog profila može biti korisno u proceni individualnog rizika za razvoj kardiometaboličkih poremećaja.

Ključne reči: biomarkeri statusa vitamina D, 25(OH)D3, $24,25(OH)_2D3$, VDMR, sdLDL, parametri proširenog lipidnog statusa