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ARTICLE

Vitamin D and cardio-metabolic biomarkers: small-scale comparative study between Libyan migrants and resident women in Serbia

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ABSTRACT

Libyan women are at high risk of vitamin D deficiency, mostly due to their lifestyle and low exposure to sun. In the last decades, Libyan residents have been forced to seek refuge in countries such as Serbia, a country with high incidence of cardio-metabolic diseases. Serbian residents tend to be deficient in vitamin D, mostly due to the lack of vitamin D fortification policy.

The aim of this study was to evaluate vitamin D status in Libyan adult women migrating to Serbia, with the assessment of cardio-metabolic and nutritional biomarkers, including erythrocytes fatty acid composition, magnesium concentration, and dietary intake. The same markers were measured in Serbian women, and comparisons between the groups were made.

Despite low vitamin D dietary intake in both study groups, we observed lower plasma vitamin D status in Libyan women. This was accompanied by a significantly lower concentration of magnesium in Libyan women. Libyan women had significantly higher omega-3 index and lower n-6/n-3 ratio in erythrocytes' phospholipids. We observed significant negative correlation between vitamin D and n-6 polyunsaturated fatty acids (PUFA) concentrations in both study groups.

Despite lower vitamin D status in the Libyan group, erythrocyte fatty acid composition, along with blood lipids' concentrations, indicated a lower cardiovascular risk. Based on our results, the discrepancy in the vitamin D status could not be ascribed to the participants' dietary intake of the micronutrient, rather is potentially associated with ethnic-specific cardio-metabolic profile, which should be confirmed in larger cohorts.

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1. Introduction

Vitamin D deficiency is becoming a global health issue, and its consequences should not be underestimated [1]. Clinical and epidemiological data report its association with a higher risk of cardiovascular and metabolic disorders, and autoimmune diseases and cancers as well [2]. Based on the current research data, Libyan population is at high risk of vitamin D deficiency, mostly due to their lifestyle and cultural customs, which particularly causes them to have inadequate skin exposure to the sun [3]. Due to the traditionally low exposure to the sun (that enables the majority of the subcutaneous synthesis of vitamin D), the status of this micronutrients among Libyan women almost exclusively relies on dietary factors and supplementation. A recent study including apparently healthy Libyan residents indicated that Libyan women (aged from 25 to 64 years) are especially prone to vitamin D deficiency with more than 80% out of 40 participants having an inadequate vitamin D status [4].

In the last decade, Libya has become a country of conflicts and unrest, and many civilians have been forced to seek refuge, mostly in European countries, such as Serbia [5]. Migrations in general can have both positive and negative impact on migrants' health. Migrants can be facing numerous health challenges associated with stress, changes in lifestyle, climate, cultural, and physical environment [6]. As literature suggests, cardio-metabolic diseases are the leading cause of disability and mortality in countries of Middle East and North Africa, including Libya [5,7]. World Health Organization categorized Libya as a country of complex emergency situations with widespread micronutrient deficiency [8]. Serbia, similarly to Libya, is a country of high incidence of cardio-metabolic diseases, with residents tending to be deficient in vitamin D, mostly due to the low dietary intake and lack of vitamin D fortification policy [9]. A recent cross-sectional study in apparently healthy Serbian adults reported that more than 60% of 170 studied participants were vitamin D deficient, with average intake far below recommended values [10]. Serbian women, on the other side, and are at risk of low vitamin D synthesis in the skin, especially in the wintertime.

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The main food sources of vitamin D are fish and shellfish, eggs, meat, dairy products or even butter and margarine [4,11,12]. Since vitamin D-rich foods also contain different types of fat, a group of researchers investigated the association between erythrocytes fatty acid composition and vitamin D status in healthy adult women [13]. The results of this study revealed no association between vitamin D status and erythrocyte fatty acid profile, even after adjusting for age and body mass index. On the contrary, a human intervention trial indicated that efficacy of vitamin D supplementation depends on the type of dietary fat. Participants of both genders were supplemented with vitamin D, while increase in vitamin D blood concentration positively correlated with the intake of monounsaturated, and negatively with the intake of polyunsaturated fatty acids [14]. Furthermore, *in vitro* data indicated that unsaturated fatty acids decrease serum levels of vitamin D by influencing vitamin D binding protein [15]. Previous studies have suggested that magnesium has an important role in the synthesis and metabolism of vitamin D as well. In a large population-based cross-sectional, high intake of magnesium (either from dietary sources or supplements) was associated with a reduced risk of vitamin D deficiency or insufficiency [16]. Another study reported a strong positive correlation between the concentrations of magnesium and vitamin D in the blood of type 2 diabetic participants [17].

The objective of our study was to evaluate vitamin D status and its relationship with dietary intake and cardio-metabolic biomarkers in apparently healthy Libyan women migrating to Serbia and living there for at least 1 year. We further compared the differences in the vitamin D status and the biomarkers between the migrant women and apparently healthy Serbian women who are lifelong residents. To assess the study objectives, we measured participants' dietary intake, plasma vitamin D status, magnesium status, serum lipid levels, and status of fatty acids in erythrocyte phospholipids of the study participants.

2. Materials and methods

2.1. Participants and study design

Presented study is a small-scale cross-sectional study, which included a total of 13 Libyan and 15 Serbian apparently healthy women (aged from 30 to 60 years; mean age 46.2 ± 8.0) not affected by medical conditions requiring pharmacological treatment. Prior to the study commencement, the participants have been residents of Belgrade at least 1 year. The recruitment was population based and performed in March of 2017 during one working week, via word-of-mouth and newspaper advertisement. The exclusion criteria were pregnancy or breast feeding, presence of pharmacologically treated chronic diseases, or presence of three or more cardio-metabolic risk factors defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III [18]. All participants gave written informed consent prior to the enrolment and study was undertaken according to the Helsinki Declaration 1983. Along with blood sampling performed by a professional nurse, anthropometric parameters were measured, together with the assessment of participants' dietary intake. All study assessments were performed in the laboratory facilities in the Centre of Research Excellence of Nutrition and Metabolism, University of Belgrade.

2.2. Anthropometric measurements

Anthropometric measurements were performed in a private room with participants' being in light clothing and without shoes. Waist and hip circumference were measured at the umbilicus and hip bones, respectively. We employed the bio-impedance analyzer, using TANITA UM072 balance (TANITA Health Equipment H.K.LTD, Hong Kong, China) to determine their weight, percentage body fat, and muscle mass. The body mass index (BMI) was calculated as weight [kg]/(height [m])^2 representing a measure of total obesity, while waist/hip and waist/height ratio represented the measures of central adiposity.

2.3. Laboratory assays

Blood samples were collected after an overnight fast into sample tubes for serum and tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Lipid status and concentration of glucose, along with other routine biochemical parameters were determined from serum samples on the same day they were collected. For this purpose, we used a clinical chemistry analyzer Cobas c111 (Roche Diagnostics, Basel, Switzerland) and Roche Diagnostics' kits according to the manufacturer's instructions. All methods for determination of the measured parameters (glucose, triglycerides, LDL-cholesterol, HDL-cholesterol and total cholesterol) involved enzymatic and colorimetric reactions. Glucose content was determined via the reaction initiated with the catalyst hexokinase, and by measuring the concentration of the end-product (NADPH) which is proportional to the glucose levels [19]. The concentration of triglycerides in the serum involved a reaction of hydrolysis of triglycerides, after which the released hydrogen peroxide reacted with 4-chlorophenol catalyzed with peroxidase and formed a red dyestuff (which intensity is directly proportional to the triglyceride concentration) [20]. LDL-cholesterol determination was based on the selective micellar solubilization of LDL-cholesterol by a non-ionic detergent and the interaction of a sugar compound and

lipoproteins; finally the generated hydrogen peroxide reacted with 4-aminoantipyrine and HSDA and forms a purple-blue dye which was proportional to the concentration of the cholesterol [21]. HDL-cholesterol measurement was based on the reaction with cholesterol esterase and cholesterol oxidase coupled with polyethylene glycol to the amino groups; the final hydrogen peroxide released after this reaction reacted with 4-amino-antypirine and HSDA and formed a purple-blue dye, proportional to the HDL-concentration [22]. Finally, total cholesterol determination is based on the determination of Δ-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol oxidase, and a subsequent measurement by the Trinder reaction of the hydrogen peroxide formed [23]. The rest of serum samples were stored at −80°C for the analyses of vitamin D and magnesium status. Samples from EDTA tubes were centrifuged to separate red blood cells from plasma. Isolated erythrocytes were washed out with cold isotonic saline and stored at −80°C for further analysis of fatty acid composition.

Vitamin D status was determined by RECIPE's HPLC Complete Kit (order no. 35000), according to manufacturer's instructions, and is described elsewhere [24]. Briefly, the precipitation and extraction agents were added to the sample and control tubes. After centrifugation, the clear supernatant was decanted into a glass vial, and placed in the sampler in the HPLC apparatus. The concentration of total vitamin D was calculated from the concentrations of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, measured by this kit. Magnesium concentration was determined by using flame atomic absorption spectrometry (AAS) on a Varian SpectrAA-10 instrument according to the previously described method [25]. Briefly, a series of mixed calibration standards were prepared. One milliliter of serum was diluted and transferred in a test tube, which is then placed in the auto sampler carousel after mild shaking. The measurement is carried out automatically, and the results are expressed as the average of two measurements according to the standard addition calibration method.

2.4. Analyses of fatty acid composition

Firstly, lipids were extracted from erythrocytes using the organic solvents chloroform and isopropanol (7:11, by volume) as previously described [26]. Further, phospholipids were separated by a thin-layer of chromatography with the mixture of petroleum ether, diethyl ether and glacial acetic acid (87: 12 : 1, by volume)) on Silica Gel GF plates (Merck, Darmstadt, Germany). According to the previously published procedure [27] with slight modifications, direct transesterification of fatty acids was carried out, followed by the evaporation of hexane extracts under a stream of nitrogen. The final residue was dissolved in hexane and injected into the Shimadzu chromatograph GC 2014. The chromatograph was equipped with a flame ionization detector and Rtx 2330 column (60m x 0.25mmID, 0.2µm, Restek).

Adequate separation of methyl esters was obtained over a 50 min period with a temperature of 140°C held for 5 min, then increased to 220°C at a rate of 3°C/min and held on final temperature for 20 min. The identification was made by comparing peak retention times with standard mixtures and the contents of fatty acids from C16:0 through C22:6n-3 were expressed as a percentage of total fatty acids identified.

The percentage of total saturated fatty acids (SFAs) was calculated as the sum of the percentages of C16:0 and C18:0, while the percentage of monounsaturated fatty acids (MUFAs) represents the sum of C16:1n-7, C18:1n-9, and C18:1n-7 percentages. The percentage of total PUFAs was calculated from the percentages of the individual polyunsaturated long-chain fatty acids C18:2n-6, C20:3n-6, C20:4n-6, C22:4n-6, C20:5n-3, C22:5n-3, and C22:6n-3, which were expressed as n-3 and n-6 PUFA separately.

2.5. Dietary intake assessment

Participants' dietary intake was evaluated by using a validated 24-hour dietary recall questionnaire at two separate days (separated by at least one week) [4]. Prior to the dietary intake section, this questionnaire contained questions about demographic data as well. A well-trained and experienced interviewer in dietary intake assessment collected information on food type, preparation methods, recipes, and commercial products from each participant in a face-to-face interview. For estimating the portion sizes, we provided pictures of various foods, dishes, and beverages, as previously reported [28]. Nutrient calculations (including total energy, macronutrients, and vitamin D intake) were performed using the European Food Safety Authority – EFSA' validated dietary assessment tool Diet Assess and Plan [29,30].

2.6. Statistical analysis

Normal distribution of the data was checked by the Shapiro-Wilk test. For normally distributed variables, the independent sample t-test was applied, while Mann-Whitney test was used to compare non-normally distributed variables. Associations of vitamin D status and other analyzed parameters were evaluated by Spearman coefficient of correlation, since vitamin D level did not follow a normal distribution. Literature data point out that age can affect vitamin D status significantly, because studies showed that

older individuals are prone to deficiency as they have lower levels of provitamin D3 in the skin and are less efficient in producing the vitamin [31]. Thus, we performed partial Spearman correlation taking into account age as controlling variable, as well as age together with either anthropometric or lipid indices as controlling variables. We employed intention-to-treat protocol for carrying out the analysis. Analyses were performed using the SPSS software (ver. 20.0) and p values < 0.05 were considered statistically significant. Normal data are presented as mean (SD) while non-normal as median [interquartile range].

3. Results

3.1. Clinical characteristics and dietary intake of the study participants

General characteristics and dietary intake of the study participants are presented in Table 1. Libyan women had significantly higher waist/height ratio (mean difference, MD = 0.062, 95%CIs: 0.006, 0.119; p = 0.030), while other anthropometric parameters did not differ. There was a significant difference in the mean age of the two groups (MD = −10.948, 95%CIs: −15.562, −6.334; p < 0.001).

Serbian women had significantly lower total energy intake (p = 0.003). The intake of vitamin D was found to be far below the latest EFSA' recommendations [32] in both study groups, with no significant difference between them.

3.2. Vitamin D, magnesium status and biochemical parameters

Between group differences in the status of vitamin D, magnesium and gluco-lipid parameters are presented in Table 1. Concentration of total serum vitamin D tended to be lower in the Libyan group in comparison with the Serbian group (p = 0.058, Table 1). The proportions of deficient participants (vitamin D lower than 50 nmol/L [33]) in Libyan and Serbian groups were 69.23% (9 of 13) and 33.33% (5 of 15), respectively. In addition, Libyan females had significantly lower (p = 0.003) concentration of magnesium.

Serbian women had significantly higher levels of total cholesterol (MD = −1.042, 95%CIs: −1.89, −0.188, p = 0.019), and HDL cholesterol (MD = −0.344, 95%CIs: −0.619, −0.069, p = 0.016) compared with the Libyan. The concentration of LDL cholesterol also tended to be higher in the Serbian group (p = 0.060). We observed no differences in parameters of liver and kidney enzyme function among the groups (data not presented).

3.3. Fatty acid composition

The results of fatty acid composition analyses are presented in Table 2. Libyan women had significantly higher levels of total polyunsaturated fatty acids (PUFA) (MD = 4.576, 95%CIs: 2.695, 6.455; t(14.489) = 5.204, p < 0.001), n-3 (MD = 2.279, 95% CIs: 1.397, 3.162; t(26) = 5.309, p < 0.001) and n-6 (MD = 2.295, 95%CIs: 0.865, 3.726; t(26) = 3.298, p = 0.003) groups, compared with Serbian women. The levels of individual fatty acids inclusive of eicosapentaenoic acid (EPA, C22:5n-3) (MD = 0.382, 95%CIs: 0.139, 0.625; t(26) = 3.232, p = 0.003), docosahexaenoic acid (DHA, C22:6n-3) (MD = 1.901, 95%CIs: 1.305, 2.498; t(26) = 6.550, p < 0.001) and arachidonic acid (C20:4n-6) (2.006, 95%CIs: 0.894, 3.119; t(28) = 3.696, p = 0.002) were significantly higher in Libyan in comparison with Serbian women. On the contrary, the ratio of n-6 to n-3 PUFA was significantly lower in Libyan group (MD = −1.789, 95%CIs: −2.687, −0.891; t(26) = −4.098, p < 0.001). Significantly higher levels of total saturated fatty acids (SFA) (MD = −3.540, 95%CIs:

Table 1. Dietary intake, characteristics of the study groups, vitamin D status and biochemical parameters.

Parameter	Libyan women	Serbian women	P value
Age (years)	40.4 ± 5.7	51.3 ± 6.1	**<0.001**
Energy (kcal/day)	2009.75 ± 486.49	1478.5 ± 357.74	**0.003**
CHO (% TE)	44.99 ± 8.61	42.41 ± 7.63	0.408
Protein (% TE)	15.68 ± 2.72	14.16 ± 3.16	0.188
Fat (% TE)	39.33 ± 2.32	43.43 ± 6.88	0.166
Vitamin D (μg/day)	1.52 [1.53]	2.51 [2.23]	0.928
BMI (kg/m^2)	29.1 ± 5.5	25.3 ± 5.0	0.075
Body weight (kg)	77.8 ± 23.8	70.2 ± 13.2	0.310
Waist (cm)	91.7 ± 11.4	84.9 ± 12.2	0.145
Waist/Hip	0.82 ± 0.05	0.80 ± 0.06	0.510
Waist/Height	0.57 ± 0.06	0.50 ± 0.07	**0.030**
Total vitamin D (nmol/L)	43.75 [36.42]	65.75 [40.29]	0.058
Magnesium (mg/dL)	1.66 [0.09]	1.81 [0.08]	**0.003**
Triglycerides (mmol/L)	0.86 ± 0.32	0.91 ± 0.41	0.757
Total chol. (mmol/L)	4.77 ± 0.70	5.81 ± 1.34	**0.019**
LDL-chol.(mmol/L)	2.93 ± 0.68	3.65 ± 1.15	0.060
HDL-chol.(mmol/L)	1.45 ± 0.36	1.79 ± 0.35	**0.016**
Glucose (mmol/L)	4.94 ± 0.44	5.04 ± 0.32	0.503

Normal data are presented as mean ± SD, while non-normally distributed as median [interquartile range]
Independent sample t test was applied for comparison of normally distributed data; Mann-Whitney test was used to compare non-normally distributed variables

Table 2. Fatty acid profile of erythrocytes' membrane phospholipids.

Fatty acid (%)	Libyan women	Serbian women	P value
Saturated	41.13 ± 2.99	44.67 ± 0.73	**<0.001**
16:0	20.36 ± 1.37	24.46 ± 0.67	**<0.001**
18:0	20.77 ± 2.22	20.21 ± 0.54	0.351
Monounsaturated	14.31 ± 0.90	15.35 ± 0.85	**0.004**
16:1n-7	0.23 ± 0.08	0.20 ± 0.10	0.418
18:1n-9	12.74 ± 1.01	13.46 ± 0.84	0.055
18:1n-7	1.34 ± 0.39	1.68 ± 0.42	**0.032**
n-6 polyunsaturated	36.84 ± 2.26	34.55 ± 1.37	**0.003**
18:2n-6	12.94 ± 1.87	12.36 ± 1.43	0.365
20:3n-6	2.03 ± 0.42	3.08 ± 0.46	**<0.001**
20:4n-6	17.47 ± 1.73	15.56 ± 1.21	**0.002**
22:4n-6	4.40 ± 0.88	3.54 ± 0.34	**0.002**
n-3 polyunsaturated	7.71 ± 1.11	5.43 ± 1.15	**<0.001**
20:5n-3	0.31 ± 0.10	0.31 ± 0.21	0.948
22:5n-3	1.76 ± 0.33	1.38 ± 0.30	**0.003**
22:6n-3	5.65 ± 0.80	3.74 ± 0.73	**<0.001**
Omega-3 index	5.96 ± 0.85	4.06 ± 0.92	**<0.001**
Total polyunsaturated	44.56 ± 3.02	39.98 ± 1.04	**<0.001**
n-6/n-3 ratio	4.85 ± 0.60	6.64 ± 1.47	**<0.001**

Data are presented as mean ± SD; Independent sample t test was applied for comparison

−5.176, −1.905; t(26) = −4.450, p < 0.001) and palmitic acid (C16:0) (MD = −4.089, 95%CIs: −4.886, −3.292; t (28) = −10.508, p < 0.001) and total monounsaturated fatty acids (MUFA) (MD = −1.034, 95%CIs: −1.717, −0.352; t(26) = −3.134, p = 0.004) were observed in Serbian women.

3.4. Correlations between vitamin D status and fatty acid composition

As presented in Table 3, we observed significant negative correlation between vitamin D status and n-6 PUFA content in both Libyan (r = 0.604, p = 0.029) and Serbian group (r = 0.579, p = 0.024). Considering the significant between-groups difference in age, we further performed partial correlation test controlling for age, and the associations remained significant. Furthermore, the associations remained significant when adjusting for either BMI or HDL along with age in both groups. When controlling for age with either waist/hip or waist/height ratio correlation remained significant only in the group of Libyan females. Only in Serbian women, the correlation remained significant when controlling for age with either total or LDL cholesterol.

4. Discussion

Average vitamin D intake for Libyan and Serbian women, was far below the proposed dietary reference values, the lower one being reported in the Libyan group. According to the latest EFSA' recommendations intake of vitamin D should be no less than 15 µg per day, while Institute of Medicine (IOM) recommends intake of 10 µg per day or more for adult women [32,34]. We also observed lower vitamin D status among Libyan women. On the other hand, selected group of Libyan women was with more favorable cardiometabolic status, as indicated through the lower serum cholesterol levels, and low n6/n3 ratio as well as higher content of individual omega-3 fatty acids in RBC membranes. Irrespective of the group, status of vitamin D was inversely correlated with omega-6 group in RBCs. Overall, our data indicate association of vitamin D status with fat metabolism in the groups of Libyan and Serbian women, irrespective of the low dietary intake of the vitamin.

Factors other than dietary intake influence the production of vitamin D in the serum, including skin pigmentation, as darker pigmentation decreases the rate of conversion of this vitamin due to the ultraviolet light from the sun [35]. However, the participants in our study were not of darker skin pigment, thus we cannot

Table 3. Spearman correlation coefficients between vitamin D status and erythrocytes' fatty acids in women from Libya and Serbia.

	VitD and n6 in Libyan women		VitD and n6 in Serbian women		VitD and n3 in Serbian women		VitD and n6/n3 ratio in Serbian women	
Controlling variable	p-value	r-value	p-value	r-value	p-value	r-value	p-value	r-value
Crude model	**0.029**	0.604	**0.024**	0.579	**0.050**	0.514	**0.050**	−0.514
Age	**0.001**	−0.631	**0.002**	−0.542	**0.011**	0.463	**0.011**	−0.463
Cholesterol + Age	0.163	−0.452	**0.026**	−0.612	0.052	0.549	**0.045**	−0.563
HDL-c + Age	**0.033**	−0.643	**0.012**	−0.671	0.084	0.498	0.075	−0.511
LDL-c + Age	0.093	−0.530	**0.048**	−0.557	0.097	0.480	0.091	−0.487
TAG + Age	0.969	−0.013	0.335	−0.291	0.147	0.426	0.147	−0.426
Waist/Hip + Age	**0.036**	−0.634	0.121	−0.473	0.248	0.362	0.248	−0.362
Waist/Height + Age	**0.038**	−0.631	0.086	−0.515	0.156	0.436	0.166	−0.427
BMI + Age	**0.038**	−0.629	**0.031**	−0.621	**0.028**	0.630	**0.030**	−0.624

suspect that this was the underlying reason which might have affected the observed difference in vitamin D status. It is more likely that cultural and lifestyle differences of the two study groups have lead to a lower vitamin D status among Libyan women.

Our results regarding the higher proportion of vitamin D deficiency among women who migrated from Libya, compared with the group of Serbia residents, is in line with the data reported from other cross-sectional studies that investigated a similar question. One cross-sectional study carried out in Netherlands, confirmed that vitamin D deficiency was more prevalent in all ethnic groups (including those from Africa and Asia), compared with the Dutch counterparts [36]. Another cross-sectional study compared the serum vitamin D in a group of 31 Middle East and African immigrants with the group of 30 of Swedish female residents. This study again confirmed that the proportion of deficient women was much higher while sufficiency was much lower in the group of female immigrant participants, compared with the group of indigenous residents of Sweden [37].

Only few studies have investigated the status of vitamin D in Libyan as well as in Serbian population. Still, the available data identify both nations as prone to vitamin D deficiency. In a recent study examining the vitamin D status in more than 400 Libyan residents, approximately 80% of the participants were vitamin D deficient (serum concentrations lower than 50 nmol/L) [4]. This study reported the highest proportion of vitamin D inadequacy in the subgroup of Libyan adult women (25–64 years old) [4]. The low vitamin D status was associated with their sedentary lifestyle, as well as the poor sun exposure, due to their cultural costumes and avoidance of short sleeves and clothes. Along with lower vitamin D status, we detected a significantly lower concentration of magnesium in Libyan women compared with Serbian ones. This was in accordance with previous studies that investigated the relation between magnesium and vitamin D levels. Gandhe et al. (2013) demonstrated a significant positive correlation between vitamin D (25-hydroxy vitamin D3) and magnesium levels in the participants with type 2 diabetes [17]. A large population-based cross-sectional study (National Health and Nutrition Examination Survey, NHANES) reported that high intake of magnesium correlated with the reduced risk of vitamin D deficiency [16]. This was explained by the magnesium's ability to enhance vitamin D bioavailability, which might be regulated by increasing the vitamin D-binding protein levels, activating the synthesis of 25-hydroxy vitamin D and by facilitating the activity of the parathyroid hormone [16].

Another important finding of our study was the between-groups difference in the fatty acid composition of erythrocytes' membranes. In comparison with Serbian women, Libyan women had a significantly higher content of total, n-3 and n-6 PUFAs. While the contents of both n-6 and n-3 PUFA were higher in Libyan women, the relative ratio of n-6 and n-3 (n-6/n-3) was significantly lower in this group. Our finding of n-6/n-3 ratio accounting for almost 7:1 among Serbian women was in accordance with the previous reports in this population [38,39]. As far as we are aware, there is no literature data reporting this parameter in Libyan women. Diets nowadays are rich in n-6 fatty acids, and, thus, have high n-6/n-3 ratio, which reflects on high n-6/n-3 ratio in erythrocytes' phospholipids. High n-6/n-3 ratio has been associated with pathogenesis of inflammatory, cardiovascular, and autoimmune diseases, as well as cancers [40]. A diet ratio of 4:1 was shown to decrease the risk of mortality from cardiovascular disease by 70% [40]. In group of Libyan females, we observed significantly higher omega-3 index, defined as a sum of erythrocyte eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) [41]. Omega-3 index is considered to be a risk factor of coronary heart disease (CHD) mortality, with values lower than 4% indicating high risk, between 4 and 8% intermediate risk and higher than 8% low risk. Based on our result on n-6/n-3 ratio and omega-3 index we could say that Libyan females were at lower risk of cardiovascular disease (CVD). In addition, our results indicated significantly higher level of total SFA, as well as palmitic acid (C16:0) in the group of Serbian females. Advisory committees, such as EFSA recommend the limitation of saturated fats in the diet, due to their association with increased CVD risk [42]. Mu et al. evaluated the association between SFA in erythrocytes phospholipids and systematic inflammation, as a risk factor of many chronic conditions, such as CVD [43]. They reported positive association between erythrocytes' total SFA, as well as palmitic (C16:0) and stearic (C18:0) content and levels of pro-inflammatory indicators among generally healthy adults.

Our analyses of vitamin D association with other measured parameters indicated a significant negative correlation between vitamin D and n-6 PUFA concentrations in both study groups. The observed correlation remained significant after adjusting for age. We found this result noteworthy considering significant between-groups difference in age.

Previous human intervention study demonstrated negative effects of PUFA intake on the success of vitamin D supplementation [16]. Another intervention trial involving 16 long-term dialysis patients, confirmed that addition of fish oil to vitamin D lowered the rate of this micronutrient compared with placebo [44]. One *in vitro* study demonstrated that unsaturated fatty acids, particularly arachidonic acid (C20:4n-6) could interrupt binding of vitamin D metabolites to vitamin D binding

protein and, thus, decrease vitamin D availability [15]. Considering these findings, lower vitamin D status among Libyan women, could be related to higher levels of total n-6 PUFA and arachidonic acid in their erythrocytes.

Between-groups comparison of blood lipids revealed a significantly lower concentration of total cholesterol in the Libyan group, with the same trend for LDL cholesterol with the difference almost reaching significance. Further on, Libyan women had significantly higher waist/height ratio, with no difference in other anthropometric indices. Applying anthropometric parameters, as additional controlling factors in the partial correlations, we noted that different factors modulate the observed correlation between vitamin D and n6 PUFA in the two groups. For instance, in Serbian women, the correlation was lost in the model additionally controlled for the indices of central obesity (waist/hip or waist/height ratio) [45,46]. This finding is in accordance with a recent cross-sectional study in healthy Serbian adults, which has identified central obesity as an independent predictor of vitamin D status, specifically in adult women [10]. Considering this, higher vitamin D status that we observed among Serbian women could be associated with the lower waist/height ratio in this group.

We are well aware of the limitations of our study, such as the low sample size, between-groups age difference and not measuring the time and duration of sun exposure. Still, considering that only few studies investigated vitamin D status among Libyan, as well as Serbian adult women, along with the lack of data on erythrocytes' fatty acid composition in the Libyan population, we find our result noteworthy.

5. Conclusion

We found lower vitamin D status in Libyan migrant women residing in Serbia in comparison with Serbian residents, but erythrocyte fatty acid composition along with blood lipids' concentrations indicated lower cardiovascular risk in the group of Libyan women. Based on our results, the discrepancy in the vitamin D status could not be ascribed to the participants' dietary intake of the micronutrient, rather is potentially associated with ethnic-specific cardio-metabolic profile, which should be confirmed in larger cohorts. We observed negative correlation between vitamin D status and n-6 PUFA content regardless of the group, indicating the link between metabolism of liposoluble vitamin D and fat metabolism. Taken together, our data add value on the raising importance of the vitamin D as promising cardio-metabolic biomarker, which however warrants further research in a larger population-specific context. In our study, intake of vitamin D was far below recommended in both groups with substantial proportion of study participants with plasma levels below 50 nmol/L, indicating necessity for enforcing public health strategies towards vitamin D food fortification, regardless of the ethnicity or specific dietary habits.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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