Gender Differences in the Association of Ferritin and 25-hydroxyvitamin D

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This study aimed to investigate gender differences in the relationship between ferritin levels and 25(OH)vitamin D deficiency in overweight persons and whether this relationship is mediated by total and visceral adiposity and fatty liver index (FLI), a marker of non-alcoholic fatty liver disease.Our study was a retrospective one in which were collecting data from 92 patients seen in an outpatient clinical centre between January 2011 and October 2017. Patients were classified with vitamin D deficiency if 25(OH)vitamin D levels were < 20.0 ng/mL. Univariate linear regression analysis assessed the association between ferritin levels and 25(OH)vitamin D deficiency, with and without correction for age, body composition (total fat mass and visceral fat mass) and FLI. In men, a statistically significant positive association between 25(OH)vitamin D deficiency and ferritin levels were found (β=0.424, p=0.008) in univariate and model adjusted for age. After adjustment for age and total fat mass and for age and visceral fat mass the association became non-significant in men (β=0.327, p=0.067 and β=0.295, p=0.106, respectively) and maintained non-significant after further adjustment for FLI (p <0.05). In women, ferritin level was negatively associated with 25(OH)vitamin deficiency in the model adjusted for age, visceral fat mass and FLI (β=-0.335, p=0.026). In this study, we showed that serum ferritin levels were negatively associated with the presence of 25(OH)vitamin D deficiency in women and this association was independent of age, body composition and FLI. No association was observed in men.

Keywords: gender differences; vitamin D; ferritin; body composition; fatty liver index

Traditionally vitamin D is associated with bone metabolism and calcium homeostasis, and its deficiency is linked to rickets in children and also osteoporosis in adults [1]. New roles have been attributed to vitamin D, and its pleiotropic effects have been shown to be attributable to immunomodulatory qualities of vitamin D receptor agonists [2]. Thus, it is not surprising that its deficiency has been linked to various conditions that have inflammation as a pathogenetic mechanism, such as autoimmune diseases, cardiovascular diseases, insulin resistance and diabetes mellitus [2-4].Accordingly, vitamin D levels are regulated by several factors, including UV exposure, dietary intake, gender and obesity [5,6].

Iron is an essential trace element for most of the physiologic processes, with a critical role in energy homeostasis, from oxygen transport to energy metabolism, but also in DNA replication and transcriptional regulation [8-10].Due to its involvement in hepatic and adipose tissue insulin resistance and oxidative stress, iron also interferes with the primary pathogenetic mechanisms of obesity-related diseases, including non-alcoholic fatty liver disease (NAFLD). Ferritin acts as the primary form of storage of iron in most cells, and small quantities are released in circulation reflecting body iron stores.Serum ferritin levels are regulated by hepcidin, which is the chief regulator of iron homeostasis [11].Hepatocytes mainly produce hepcidin, but small quantities are also provided by adipose tissue, macrophages and pancreatic cells [12-15], and its production appears to be influenced by oestrogens by regulation of ferroportin expression [16].

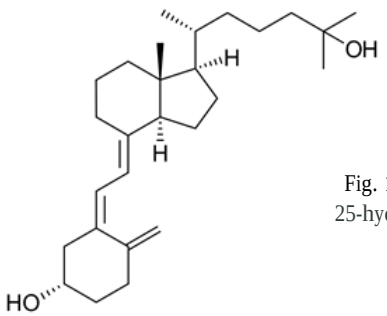

Fig. 1. Chemical formula of 25-hydroxycholecalciferol [7]

Researchers on the relation between ferritin and vitamin D levels are limited, and results are conflicting, with studies showing either a positive association [18] or no association [19,20]. Furthermore, insufficient data are available on the gender influence on this association, with one study

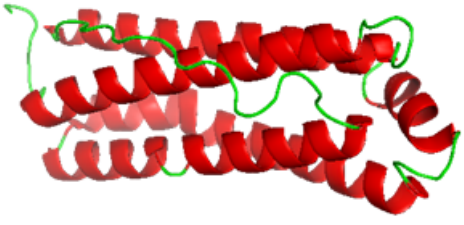

Fig. 2. The Crystallographic structure of mitochondrial ferritin [17]

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showing a positive correlation in women and but not in men [21] and a second one showing an inverse association of ferritin levels with vitamin D levels in men and a positive association in premenopausal women [22].

Our study proposed to investigate gender differences in the relationship between ferritin levels and 25(OH)vitamin D deficiency in overweight persons and whether this relationship is mediated by total and visceral adiposity and fatty liver index (FLI), a marker of NAFLD.

Experimental part

Materials and methods

Study participants

This was a retrospective study in which were collected data from charts of patients seen in an outpatient clinical centre in Bucharest, Romania between January 2011 and October 2017. Were included adults below 79 years of age, with overweight or obesity as defined by a body mass (BMI) ≥ 25 kg/m^2, with 25(OH)vitamin D levels available and which had not undergone a nutritional intervention in the previous 12 months. Patients were excluded if they had parathyroid pathology, had used vitamin D supplements in the last six months, were using hepatoprotective drugs, had a previous diagnosis of osteoporosis, gastrointestinal or autoimmune diseases, kidney or hepatic failure. Also, were excluded pregnant or lactating women and patients of non-Caucasian race.

The research was conducted following Good Clinical Practice Guidelines and the Declaration of Helsinki, and the Institutional Review Board approved the study protocol. Due to retrospective design, according to local regulations, the signature of the informed consent was not required [23].

Data collected

Detailed medical history, age, gender, results of anthropometrical measurements (weight, height, waist circumference), clinical assessments (blood pressure values and body fat composition), and results of investigations were collected from patients' files. According to local procedures, height and weight were measured in the morning, in fasting condition, with patients wearing light clothes and no shoes. BMI was calculated as weight (kg)/height^2 (m). The waist circumference was measured in standing position at a half distance between the lowest rib and the iliac crest using a soft tape. Blood pressure was measured in sitting position after 5 minutes of rest. Visceral fat mass (VFM) and body fat mass (BFM) were measured by bioelectric impedance, using Omron body composition monitor (Omron Healthcare Europe BV). Diabetes was diagnosed if fasting plasma glucose ≥126 mg/dL on two different occasions and/or HbA1c was ≥6.5% or patients had a previous diagnosis of diabetes or were following therapy with hypoglycaemiant drugs. Hypertension was defined as a systolic blood pressure of ≥140 mmHg, a diastolic blood pressure ≥ 90 mmHg, and/or use of antihypertensive therapy. Fatty liver index (FLI) [24] assessing liver steatosis was calculated with the following formula: $FLI=[e^{0.953\times\log e\,(TG)+0.139\times BMI+0.718\times\log e\,(GGT)+0.053\times\text{waist circumference}-15.745})]/[1+e^{0.953\times\log e\,(TG)+0.139\times BMI+0.718\times\log e\,(GGT)+0.053\times\text{waist circumference}-15.745}]\times 100$.

Laboratory assessments

Laboratory investigations on which data were collected included: fasting blood glucose, glycated haemoglobin (HbA1c), triglycerides, total cholesterol, HDL-cholesterol, ferritin, 25(OH)vitamin D, total calcium, magnesium, haemoglobin, haematocrit, mean corpuscular volume, iron and full iron binding capacity. As per institutional procedures, all blood samples were collected in the morning, in fasting condition and assessed in an institutional laboratory in the day of collection. HbA1c was determined using ion-exchange chromatography. Fasting plasma glucose, triglycerides, total and HDL-cholesterol were determined by routine enzymatic methods. LDL-cholesterol was calculated using the Friedewald formula [25]: LDL-cholesterol = total cholesterol - HDL-cholesterol – triglycerides/5. Haemoglobin, haematocrit and mean corpuscular volume were assessed using flow cytometry methods and iron and total iron binding capacity (TIBC) by colourimetric methods. Serum ferritin and 25(OH)vitamin D levels were measured using an electrochemi-luminescence method. Patients were classified as having vitamin D deficiency if 25(OH)vitamin D levels were <20.0 ng/mL [23].

Statistical analysis

Statistical analysis was performed using SPSS-PC 20.0 (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov tests, skewness and kurtosis, were used to evaluate the distribution of all quantitative variables. Data are presented as number (percentage, %) for categorical variables and mean ± standard deviation (SD) or median (quartile 1; quartile 3) for continuous variables. Comparing variables between groups with and without 25(OH) vitamin D deficiency and gender were performed by Student t-test, Mann-Whitney U test and chi-square test. Univariate linear regression analysis assessed the association between ferritin levels and 25(OH)vitamin D deficiency, with and without correction for age, body composition (total fat mass and visceral fat mass) and FLI. Because ferritin had a non-Gaussian distribution, for the inclusion in the regression analysis as a dependent variable, it was logarithmically transformed. The presence of collinearity among predictors was tested in all regression models employing more than one predictor. Due to collinearity issues, total fat mass and visceral fat mass were included in separate regression models.

Results and discussions

Data of 148 patients fulfilling the inclusion criteria and without exclusion criteria admitted between January 2011 and October 2017 were collected. Of these, 56 had no ferritin evaluation and were excluded from the analysis. Thus, here we present data from 92 patients with overweight or obesity and with age ranging between 20 and 69 years. The characteristics of the analysed population are presented in table 1.BMI ranged between 25.5 kg/m2and 56.0 kg/m^2, with a mean value of 32.8 kg/m^2. Of the patients analysed, 21 (22.8%) had arterial hypertension, and 9 (9.8%) had type 2 diabetes. Median levels of ferritin were 82.5μg/L, and median 25(OH)vitamin D levels were 22.5 ng/mL. Mean haemoglobin levels were 14.2 mg/dL, median iron levels 16.9μg/L and TIBC 66.2 μg/dL. According to 25(OH)vitamin D levels, 35 patients (38.0% of the sample included in the analysis) had 25(OH)vitamin D deficiency. No statistically significant difference between groups with and without 25(OH)vitamin D deficiency was observed for any parameters analysed. Median ferritin levels were100.0 μg/L in those with 25(OH)vitamin D deficiency and 68.4μg/L in those without (p=0.088).

54 patients, representing 58.7%, were women. Compared to women, men had significantly higher levels of ferritin: 174.5 μg/L *vs* 48.1 μg/L, p <0.001. Also, weight (p <0.001), BMI (p=0.048), waist circumference (p=0.002), visceral fat mass (p <0.001), diastolic blood pressure (p=0.039), fasting plasma glucose (p=0.018),

Table 1

CLINICAL CHARACTERISTICS OF PATIENTS ENROLLED (WHOLE SAMPLE AND ACCORDING TO 25(OH)VITAMIN D STATUS)

	Total N=92	25(OH)vitamin D <20 ng/ml N=35	25(OH)vitamin D ≥20 ng/ml N=57	p-value
Women, n (%)	54 (58.7%)	20 (57.1%)	34 (59.6%)	0.813
Age, years	42.3 ±12.5	44.1±13.8	41.2±11.7	0.296
Weight, kg	96.7±21.9	98.9±24.1	95.4±20.5	0.457
BMI, kg/m^2	32.8±6.1	34.5±7.0	31.8±5.3	0.040
Waist circumference, cm	106.7±14.4	108.0±13.8	106.0±14.9	0.516
Total fat mass, kg	40.2±7.3	41.5±7.5	39.6±7.2	0.306
Fat-free mass, kg	26.4±4.0	25.9±3.9	26.6±4.1	0.513
Visceral fat mass, kg	12.7±6.2	14.9±7.0	11.7±5.6	0.037
SBP, mmHg	125.3±18.9	123.6±18.1	126.4±19.5	0.497
DBP, mmHg	76.9±13.2	75.1±12.5	78.0±13.6	0.317
FPG, mg/dl	93.0 (87.0; 103.0)	94.0 (88.0; 107.0)	93.0 (87.0; 102.0)	0.538
HbA1c, %	5.5 (5.2; 5.7)	5.5 (5.4; 5.9)	5.5 (5.2; 5.7)	0.187
Total cholesterol, mg/dl	207.8±41.1	218.3±38.5	202.9±41.6	0.122
HDL-cholesterol, mg/dl	47.8±15.4	49.0±15.9	47.3±15.2	0.662
LDL-cholesterol, mg/dl	134.3±36.6	139.6±28.5	131.8±39.9	0.383
Triglycerides, mg/dl	130.0±64.4	148.1±75.3	121.3±57.1	0.081
HBP, n (%)	21 (22.8%)	9 (25.7%)	12 (21.1%)	0.605
Diabetes type 2, n (%)	9 (9.8%)	5 (14.3%)	4 (7.0%)	0.255
Total calcium, mg/dl	9.4 (9.2; 9.6)	9.4 (9.2; 9.5)	9.3 (9.2; 9.6)	0.702
Magnesium, mg/dl	2.0 (1.9; 2.1)	2.0 (1.9; 2.1)	2.0 (1.9; 2.1)	0.796
Haemoglobin, g/dl	14.2±1.5	14.2±1.4	14.2±1.5	0.869
Haematocrit, %	43.3±4.1	43.6±3.8	43.1±4.2	0.631
MCV, fl	90.7 (87.6; 94.2)	91.3 (88.6; 93.4)	89.9 (87.0; 94.7)	0.529
Iron, µg/dl	16.9 (13.2; 22.5)	16.4 (13.6; 20.3)	16.9 (12.9; 22.8)	0.744
TIBC, µg/dl	66.2 (59.1; 76.2)	64.4 (59.1; 88.0)	66.9 (60.3; 75.3)	1.00
Ferritin, µg/l	82.5 (38.6; 195.7)	100.0 (49.0; 258.4)	68.4 (37.9; 171.0)	0.088
FLI	74.0±25.0	80.3±23.1	68.0±25.5	0.042

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HbA1c = glycated haemoglobin; HBP = high blood pressure; MCV = mean corpuscular volume; TIBC = total iron binding capacity; FLI = fatty liver index

haemoglobin ($p < 0.001$) and haematocrit ($p < 0.001$) were higher in men than in women. BMI was 34.3 kg/m^2 in men and 31.8 kg/m^2 in women; visceral fat mass was 17.4 kg in men and 9.1 kg in women. FLI score was also significantly higher in men than in women: 88.4 vs 63.2, $p < 0.001$. Total fat mass was higher in women than in men (43.5 kg vs 36.0 kg, $p < 0.001$). No difference was observed in the 25(OH)vitamin D levels and the frequency of 25(OH)vitamin D deficiency between the genders. The frequency of 25(OH), vitamin D deficiency, was 39.5% in men and 37% in women ($p = 0.813$, table 2).

In men, serum ferritin levels were significantly higher in those with 25(OH)vitamin D deficiency compared to those without 261.7 (159.5; 415.0) vs 146.8 (63.7; 192.8), $p = 0.006$. In women, the difference observed between those with and without 25(OH)vitamin D deficiency was not statistically significant: 56.6 (25.3; 112.1) in those with 25(OH)vitamin D deficiency and 46.7 (27.4; 96.9) in those without 25(OH)vitamin D deficiency, $p = 0.788$.

A univariate linear regression analysis was performed with Log ferritin as dependent variable and presence of 25(OH)vitamin D deficiency as an independent variable. Statistically significant positive association between 25(OH)vitamin D deficiency and ferritin levels was found only in men ($\beta = 0.424$, $p = 0.008$) for this model. The adjustment for age did not change the significance of the association. After adjustment for age and total fat mass (Model 1) and for age and visceral fat mass (Model 2) the association between ferritin and 25(OH)vitamin D deficiency become non-significant in men ($\beta = 0.327$, $p = 0.067$ and $\beta = 0.295$, $p = 0.106$, respectively) and maintained non-significant after further adjustment for FLI ($p < 0.05$). In women, no association was observed neither in the unadjusted model nor after the adjustment for age and total fat mass or age and visceral fat mass. However, in women, Log ferritin level was negatively associated with 25(OH)vitamin deficiency in the model adjusted for age, visceral fat mass and FLI ($\beta = -0.335$, $p = 0.026$, table 3). For all adjusted models Variance Inflation Factor was <10 and correlation coefficients <0.80, showing no multicollinearity in the data.

In this retrospective research, we evaluated the gender differences in the relationship between ferritin levels and 25(OH)vitamin D deficiency in overweight men and women and whether body composition and FLI mediated

	Men N=38	Women N=54	p-value
Age, years	42.5±13.7	42.2±11.8	0.914
Weight, kg	110.1±17.1	87.3±19.9	<0.001
BMI, kg/m^2	34.3±5.3	31.8±6.5	0.048
Waist circumference, cm	112.2±11.3	102.9±15.2	0.002
Total fat mass, kg	36.0±5.9	43.5±6.6	<0.001
Fat-free mass, kg	28.8±3.9	24.4±2.8	<0.001
Visceral fat mass, kg	17.4±5.4	9.1±3.9	<0.001
SBP, mmHg	128.7±14.4	122.9±21.3	0.144
DBP, mmHg	80.3±9.6	74.5±14.9	0.039
FPG, mg/dl	97.0 (90.0; 107.0)	91.0 (86.0; 97.0)	0.018
HbA1c, %	5.5 (5.3; 5.7)	5.5 (5.2; 5.7)	0.363
Total cholesterol, mg/dl	208.0±38.0	207.7±43.4	0.977
HDL-cholesterol, mg/dl	42.2±14.0	51.6±15.2	0.007
LDL-cholesterol, mg/dl	136.3±35.5	133.0±37.6	0.699
Triglycerides, mg/dl	146.2±52.3	118.7±69.9	0.059
HBP, n (%)	11 (28.9%)	10 (18.5%)	0.241
Diabetes type 2, n (%)	5 (13.2%)	4 (7.4%)	0.361
Total calcium, mg/dl	9.4 (9.2; 9.6)	9.3 (9.1; 9.5)	0.100
Magnesium, mg/dl	2.0 (1.9; 2.1)	2.0 (1.9; 2.1)	0.248
Haemoglobin, g/dl	15.4±0.8	13.4±1.2	<0.001
Haematocrit, %	46.6±2.7	41.0±3.2	<0.001
MCV, fl	92.5 (88.6; 94.8)	89.7 (86.7; 93.4)	0.118
Iron, µg/dl	18.6 (13.6; 21.6)	15.0 (12.4; 23.2)	0.420
TIBC, µg/dl	75.3 (67.8; 88.0)	62.4 (57.3; 66.9)	0.007
Ferritin, µg/l	174.5 (84.3; 261.7)	48.1 (27.4; 96.9)	<0.001
FLI	88.4±11.5	63.2±26.9	<0.001
25-hidroxyvitamin D, ng/ml	22.4 (16.4; 32.0)	24.8 (16.2; 29.4)	0.794
25(OH) vitamin D deficiency, n (%)	15 (39.5%)	20 (37.0%)	0.813

Table 2
CLINICAL CHARACTERISTICS OF PATIENTS ENROLLED ACCORDING TO THEIR GENDER

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HbA1c = glycated haemoglobin; HBP = high blood pressure; MCV = mean corpuscular volume; TIBC = total iron binding capacity; FLI = fatty liver index

Table 3
ASSOCIATION OF SERUM FERRITIN LEVELS WITH 25(OH)VITAMIN D DEFICIENCY BY GENDER

	Men β (p-value)	Women β (p-value)
Unadjusted model	**0.424 (0.008)**	0.023 (0.868)
Model 1	**0.345 (0.032)**	0.039 (0.768)
Model 2	0.327 (0.067)	-0.130 (0.419)
Model 3	0.295 (0.106)	-0.097 (0.495)
Model 4	0.127 (0.541)	-0.309 (0.083)
Model 5	0.135 (0.518)	**-0.335 (0.026)**

Unadjusted model included only ferritin as dependent variable and 25(OH)vitamin D deficiency as predictor. Normal 25(OH)vitamin D levels were used as reference. Model 1: adjusted for age, Model 2: adjusted for age and total fat mass, Model 3: adjusted for age and visceral fat mass, Model 5: adjusted for age, total fat mass and fatty liver index, Model 5: adjusted for age, visceral fat mass and fatty liver index

this relationship. After adjustment for age, body composition parameters and FLI score, serum ferritin levels were negatively associated with the presence of 25(OH)vitamin D deficiency in women; no association was observed in men.

Data on the gender differences in the relationship between ferritin levels and vitamin D levels are limited. We were able to identify only two publications investigating these differences and both emerged from researchers performed in Korean populations [21,22] and reported results are contradictory. In an analysis of data of 695 persons Jeong et al. [21] reported no correlation between ferritin and 25(OH)vitamin D levels in men and a positive relation between ferritin and 25(OH)vitamin D levels in women, with higher ferritin levels in those with higher vitamin D levels [21]. The second study, enrolling participants from the 2012 Korean National Health and Nutrition Examination Survey (KNHANES) showed an inverse association between ferritin and 25(OH)vitamin D levels in men and a positive association in premenopausal women, with lower ferritin levels in those with 25(OH)vitamin D deficiency and insufficiency as compared

to those with normal levels of this vitamin [22]. Our results are in line with the former study with regards to lack of relationship in men; we also observed similar results to the latter study in women, showing that in women the presence of 25(OH)vitamin D deficiency was associated with lower serum ferritin levels[26] The differences observed in the direction of the association among the studies may be due to the variables used for the adjustment – only age in the article of Jeong at al. [21] (positive association also seen in our results when the correction for made just for age) and multiple parameters, some of which are also included in the FLI score we used in the article of Seong et al. [22].

The observed gender differences in the associations may be due to the oestrogen and testosterone levels which have been associated with both ferritin and vitamin D [5,16].Pregnancy and ovulation, conditions in which oestrogen production increases, were shown to be associated with higher 25(OH)vitamin D levels [27,28].Vitamin D stimulates 17β-oestradiol synthesis, consequent vitamin D receptors expression and thus oestrogen signalling [29]. In men vitamin D metabolising enzymes (CYP2R1, CYP27B1, and CYP24A1) and the vitamin D receptors are expressed in the male reproductive system, including Leydig cells, thus suggesting a link between sex steroid productions and vitamin D [30].Oestrogen shave recently emerged as factors involved in iron metabolisms. In a cohort from 1990-2000 NHANES, it was shown that hormonal replacement therapy was associated with lower ferritin levels (β=-34.13, p=0.0002), independent of other potential confounders such as breakthrough bleeding and hysterectomy [31]. The link hypothesised is the influence of oestrogens on the hepcidin synthesis, the primary regulator of iron homeostasis [32].

In men, ferritin levels were positively associated with the presence of 25(OH)vitamin D deficiency in the unadjusted model. After adjustment for total and visceral fat mass the association lost its statistical significance, suggesting that body composition mediated the relationship. Obesity has been shown to be influenced both vitamin D and ferritin levels [21,33,34].A metanalysis including 34 cross-sectional studies showed a significant but weak correlation between BMI and 25(OH)vitamin D levels, with a 4% reduction in 25(OH)vitamin D with each 10 % increase in BMI [6].Among conditions hypothesised in the association between obesity and vitamin D levels are 25(OH)vitamin D sequestration in the adipose tissue [35] and volumetric dilution of vitamin D [36]. Also, obesity is a state of subclinical chronic inflammation associated with increased production of pro-inflammatory cytokines. In this context of chronic inflammation vitamin D may act as an acute phase reactant and with consequent decreased circulating levels of 25(OH)vitamin D [33].Visceral and subcutaneous adipose tissue has also been associated with ferritin levels [37] and, as both adiposity and increased hepatic iron stores have been linked with states of insulin resistance and increased fasting insulin and glucose levels, one of the hypothesised mechanisms is increased insulin resistance [38]. These mechanisms may explain our results observed in men.

In women, we found that the association between ferritin and 25(OH)vitamin D was not mediated by the total or visceral adiposity. After adjustment for FLI score, the association become statistically significant with lower ferritin levels in those with vitamin D deficiency. Previously it was showed that one-third of the patients with NAFLD, the hepatic expression of metabolic syndrome, have higher ferritin levels, with hepatic iron deposition [11].Also, it was showed that iron stores were associated with higher levels of sex hormone binding globulin and lower testosterone levels in men [39] and women with PCOS [40].We did not assess testosterone and oestrogen levels in our group of patients.Thus we can only speculate that the potential causes of our observations just in women may be represented by the endocrinological effect of iron stores in NAFLD, which were linked to sex binding globulin hormones level [41] and with consecutive lower availability of oestrogen levels [40,42]. Additionally, while in men obesity was associated with lower testosterone levels, in women obesity was associated with lower oestrogen levels in fertile women and higher oestrogen levels in postmenopausal women [43-45].

Our research has several limitations that should be discussed.[46,47]. First, due to its retrospective design, we cannot evaluate the causality - we cannot assess whether 25(OH)vitamin D deficiency causes lower ferritin levels of vice versa. Secondly, we enrolled a small sample size of overweight and obese patients; thus, our findings are limited to this population. Although our study has these limitations, this is the first reported study assessing gender differences in the relationship between ferritin and vitamin D in a Caucasian population.

In conclusion, in this study, we showed that serum ferritin levels were negatively associated with the presence of 25(OH)vitamin D deficiency in women and this association was independent of age, body composition and FLI. No association was observed in men. Further studies on larger samples, also evaluating oestrogen and testosterone levels are warranted to confirm our findings.

References

1.SUDA, T., UENO, Y., FUJIIK SHINKI T., Vitamin D and bone. J Cell Biochem. 2003;88:259H266.

2.CRESCIOLI C., MINISOLA S., Vitamin D: Autoimmunity and Gender. Curr Med Chem. 2017;24:2671H2686.

3.VASILE M., CORINALDESI C., ANTINOZZI C., et al. Vitamin D in autoimmune rheumatic diseases: A view inside gender differences. Pharmacol Res. 2017;117:228H241.

4.VLACHOPOULOS C., ROKKAS K., IOAKEIMIDIS N., et al., Inflammation, metabolic syndrome, erectile dysfunction, and coronary artery disease: common links. Eur Urol. 2007;52:1590H1600.

5.BOUILLON R., OKAMURA W.-H., NORMAN A.-W., Structure-function relationships in the vitamin D endocrine system. Endocr Rev. 1995;16:200H257.

6.SANEEI P., SALEHI-ABARGOUEI A., ESMAILLZADEH A., Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. Obes Rev. 2013;14:393H404.

7.*** https://en.wikipedia.org/wiki/Calcifediol accessed Nov 2017

8.SHEFTEL A., STEHLING O., LILL R., Iron-sulfur proteins in health and disease. Trends Endocrinol Metab., 2010;21:302H314.

9.GOZZELINO R., JENEY V., SOARES M.-P., Mechanisms of cell protection by heme oxygenase-1. Annu Rev PharmacolToxicol., 2010;50:323H354.

10.EVSTATIEV R., GASCHE C., Iron sensing and signalling. Gut, 2012;61:933H952.

11.DATZ C., MÜLLER E., AIGNER E., Iron overload and non-alcoholic fatty liver disease. Minerva Endocrinol., 2017;42:173H183.

12.NICOLAS G., CHAUVET C., VIATTE L., et al., The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest. 2002;110:1037H1044.

13.NGUYEN N.-B., CALLAGHAN K.-D., GHIO A.-J., et al., Hepcidin expression and iron transport in alveolar macrophages, Am J Physiol Lung Cell Mol Physiol., 2006;291: L417HL425.

14.BEKRI S., GUAL P., ANTY R., et al., Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology, 2006;131:788H796.

15.AIGNER E., FELDER T.-K., OBERKOFLER H., et al., Glucose acts as a regulator of serum iron by increasing serum hepcidin concentrations, J Nutr. Biochem. 2013;24:112H117.
16.QIAN Y., YIN C., CHEN Y., et al., Estrogen contributes to regulating iron metabolism through governing ferroportin signaling via an estrogen response element. Cell Signal., 2015;27:934H942.
17.*** https://en.wikipedia.org/wiki/Mitochondrial_ferritin, accessed Nov 2017
18.SIM J.-J., LAC P.-T., LIU I.-L., et al., Vitamin D deficiency and anemia: a cross-sectional study, Ann Hematol. 2010;89:447H452.
19.MONLEZUN D.-J., CAMARGO C.-A, MULLEN J.-T., et al., Vitamin D Status and the Risk of Anemia in Community-Dwelling Adults: Results from the National Health and Nutrition Examination Survey 2001-2006, Medicine (Baltimore), 2015;94:e1799.
20.SMITH E.-M., ALVAREZ J.-A., MARTIN G.-S., et al., Vitamin D deficiency is associated with anaemia among African Americans in a US cohort, Br J Nutr. 2015;113:1732H1740.
21.JEONG D.-W., LEE H.-W., CHO Y.-H., et al., Comparison of serum ferritin and vitamin D in association with the severity of nonalcoholic fatty liver disease in Korean adults. Endocrinol Metab (Seoul), 2014;29:479H488.
22.SEONG J.-M., YOON Y.-S., LEE K.-S., et al., Gender difference in relationship between serum ferritin and 25-hydroxyvitamin D in Korean adults, PLoS One. 2017;12:e0177722.
23.MIHAI D., BRATILA E., MEHEDINTU C., BERCEANU C., PITURU S.M., The ethical aspects regarding cryopreserved embryos, Romanian Journal of Legal Medicine, 2017 25: 3 : 317-321
24.BEDOGNI G., BELLENTANI S., MIGLIOLI L., et al., The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population, BMC Gastroenterol., 2006;6:33.
25.FRIEDEWALD W.T., LEVY R.I., FREDRICKSON D.S., Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin Chem. 1972;18:499H502.
26.*** INSTITUTE OF MEDICINE. Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): The National Academies Press; 2011.
27.GRAY T.K., MC ADOO T., HATLEY L., et al. Fluctuation of serum concentration of 1,25-dihydroxyvitamin D3 during the menstrual cycle, Am J Obstet Gynecol. 1982;144:880H884.
28.ELENKOV I.J., WILDER R.L., BAKALOV V.K., et al., IL-12, IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times, J Clin Endocrinol Metab. 2001;86:4933H4938.
29.NASHOLD F.E., SPACH K.M., SPANIER J.A., et al., Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression, J Immunol. 2009;183:3672H3681.
30.BLOMBERG JENSEN M., NIELSEN J.E., JØRGENSEN A., et al., Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract, Hum Reprod., 2010;25:1303H1311.
31.MILLER E.M., Hormone replacement therapy affects iron status more than endometrial bleeding in older US women: A role for estrogen in iron homeostasis?, Maturitas, 2016;88:46H51.
32.GANZ T., NEMETH E., Iron sequestration and anemia of inflammation, Semin Hematol. 2009;46:387H393.
33.POURSHAHIDI K.L., Vitamin D and obesity: current perspectives and future directions, Proc Nutr Soc. 2015;74:115H124.
34.EARTHMAN C.P., BECKMAN L.M., MASODKAR K., et al., The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications, Int J Obes (Lond), 2012;36:387H396.
35.WORTSMAN J., MATSUOKA L.Y., CHEN T..C, et al., Decreased bioavailability of vitamin D in obesity, Am J ClinNutr. 2000;72:690H693.
36.DRINCIC A.T., ARMAS L.A., VAN DIEST E.E., et al., Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity, Obesity (Silver Spring), 2012;20:1444H1448.
37.IWASAKI T., NAKAJIMA A., YONEDA M., et al., Serum ferritin is associated with visceral fat area and subcutaneous fat area, Diabetes Care, 2005;28:2486H2491.
38.FERNANDEZ-REAL J.M., RICART-ENGEL W., ARROYO E., et al., Serum ferritin as a component of the insulin resistance syndrome, Diabetes Care 1998;21:62H68.
39.LIU Z., YE F., ZHANG H., et al., The association between the levels of serum ferritin and sex hormones in a large scale of Chinese male population, PLoS One, 2013;8:e75908.
40.MARTINEZ-GARCIA M.A., LUQUE-RAMIREZ M., SAN-MILLAN J.L., et al., Body iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance, and genomic variants related to inflammation, oxidative stress, and iron metabolism, Diabetes Care, 2009;32:1525H1530.
41.GAUTIER A., LAINE F., MASSART C., et al., Liver iron overload is associated with elevated SHBG concentration and moderate hypogonadotrophic hypogonadism in dysmetabolic men without genetic haemochromatosis, Eur J Endocrinol, 2011;165:339H343.
42.FUI M.N., DUPUIS P., GROSSMANN M., Lowered testosterone in male obesity: mechanisms, morbidity and management, Asian J Androl. 2014;16:223H231.
43.FREEMAN E.W., SAMMEL M.D., LIN H., et al., Obesity and reproductive hormone levels in the transition to menopause, Menopause, 2010;17:718H726.
44.BALMUS I.M., CIOBICA A., ANTIOCH I., et al., Oxidative Stress Implications in the Affective Disorders: Main Biomarkers, Animal Models Relevance, Genetic Perspectives, and Antioxidant Approaches, Oxidative Medicine And Cellular Longevity, Article Number: 3975101,2016
45.BARBU C.G., ARSENE A.L., FLOREA S., ALBU A., SIRBU A., MARTIN S., NICOLAE A.C., BURCEA DRAGOMIROIU G.T., POPA D.E., VELESCU B.S., DUMITRESCU I.B., MITREA N., DRAGANESCU D., LUPULIASA D., SPANDIDOS D.A., TSATSAKIS A.M., DRAGOI C.M., FICA S. Cardiovascular risk assessment in osteoporotic patients using osteoprotegerin as a reliable predictive biochemical marker, Molecular Medicine Reports, 16(5), 6059-6067, 2017.
46. HAINAROSIE, R., PITURU, S., STEFANESCU, D.C., HAINAROSIE, M., IONITA, I., PIETROSANU, C., IONUT, G., ZAINEA, V., Methylene Blue Staining Test in Assessing Safe Margins in Laryngeal Papillomatosis, Rev. Chim. (Bucharest) ,**68**, no. 11, 2017, p. 2731
47. STEFANESCU, D.C., CEACHIR, O., ZAINEA, V., HAINAROSIE, M., PIETROSANU, C., IONITA, I.G., HAINAROSIE, R., The Use of Methylene Blue in Assessing Disease Free Margins During CO2 LASER Assisted Direct Laryngoscopy for Glottis Cancer, Rev. Chim. (Bucharest),67, no. 7, 2016, p. 1327

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