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ORIGINAL ARTICLES AND REVIEWS

Association between serum vitamin D and metabolic syndrome in middle-aged and older adults and role of supplementation therapy with vitamin D

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Abstract

Objectives. To evaluate i) the correlation between vitamin D (vit. D) serum concentrations and metabolic syndrome (MetS); ii) the efficacy of 6 months supplementation therapy with vit. D.

Method. 200 patients were enrolled. Blood analyses and anthropometric measurements were carried out. Patients with hypovitaminosis D received an oral supplement therapy.

Results. 81% of the sample shows vit. D levels < 30 ng/mL. Rate of MetS was significantly higher in vit. D deficiency group than in vit D insufficiency ($p = 0.009$) and sufficiency ($p = 0.002$) groups. Vit. D shows a significant negative correlation with both waist circumference (WC) (ρ - 0.202 $p = 0.004$) and glycaemia values (FBG) (ρ -0.185 $p = 0.009$). After the supplementation therapy in a group of 60 subjects a significant increase in vit. D levels ($p = 0.001$) and a significant reduction in WC values ($p = 0.001$) were observed.

Conclusions. MetS, WC and FBG appeared to be associated vit. D status and it is well-known that central obesity, with the inflammatory alterations thereto correlated that determine insulin resistance, can be considered the "primum movens" for the development of MetS.

Key words

- vitamin D
- elderly
- supplementation
- metabolic syndrome
- cardiovascular risk

INTRODUCTION

Recent scientific developments confirm that vitamin D (vit. D) plays a relevant role in controlling osteoarticular homeostasis, as well as in prevention and, in some cases, in treatment of pathologies affecting other organs and apparatuses, assuming its possible pleiotropic action [1].

It is interesting to note a possible correlation between low vit. D levels and an increased cardiovascular risk that should therefore be investigated [2, 3]. Osteoporosis and atherosclerosis are frequently diagnosed in geriatrics and a possible correlation between these conditions was recently assumed [4]. Hypovitaminosis D, whose incidence increases with age, may represent an interesting risk factor common to the reduction of bone mass as well as of the atherosclerotic process [5]. Moreover, several scientific evidences support the hypothesis of a possible association between low vit. D levels and obesity, arterial hypertension, intolerance to glucose and dyslipidemia [3, 5-10]. Furthermore, a number of studies have investigated a possible correlation between hypovitaminosis D and metabolic syndrome (MetS), a condition characterized by the contemporary presence of central obesity, arterial hypertension and altered lipidic and glycide metabolism [11-13]. Nonetheless, results are often contradictory [14, 15]. Several studies showed that lower 25(OH)D concentrations are independently associated with an increased likelihood of MetS [11, 13, 16]. A recent publication has shown that in elderly patients a major serum level of vit. D is associated to a lesser prevalence of MetS, dyslipidemia, abdomen obesity and hyperglycaemia [17]. Contrariwise, other authors failed to find an association of these factors [3, 14, 15]. These conflicting findings may be due to differences in the characteristics of the study populations, such as age, prevalence of cardio-metabolic risk factor, proportion of overweight or obese individuals, ethnicity and lifestyle. The different methods used for

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determination of 25[OH]D may be a further source of confusion. Moreover, most of the results were not adjusted for medications and serum intact parathyroid hormone (PTHi).

The primary aim of this study is to evaluate a possible correlation between 25-hydroxyvitamin D (25(OH)D) serum concentrations, the best biomarker of vitamin D status [18], and MetS in elderly subject, so far not overly considered, as well as in middle-aged adult subjects in whom the presence of alterations typical of MetS is more common.

The importance of keeping sufficient plasma levels of vit. D in order to implement primary prevention of falls and fractures is a matter of fact [19]. Furthermore, the possible role of vit. D supplementation therapy in controlling the typical alterations of MetS has not yet been thoroughly analyzed. A recent meta-analysis showed that supplementation of vit. D is effective in preventing overall mortality in a long-term treatment [20]. A secondary aim of the study is to evaluate the efficacy of an oral supplementation therapy in subjects with hypovitaminosis D evaluating its efficacy as regards reaching adequate serum vit. D levels as well as a possible control of the principal risk factors of MetS.

MATERIALS AND METHODS

Community-dwelling subjects who visited the ambulatory of the Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences of Sapienza University of Rome between March and June 2014, for evaluation of cardiovascular risk factors, were considered for this study.

Exclusion criteria were: a long history of osteoporosis; current treatment with vit. D, calcium supplements, other drugs for the treatment of osteoporosis/osteopenia; active gastroenteric pathology.

The study was conducted according to the guidelines on biomedical research involving human subjects (Declaration of Helsinki) and was approved by the "Science of Aging" Interdepartmental Research Center of Sapienza University of Rome. Informed consent from each patient was obtained.

A clinical interview investigated medical history, home therapy and general habits of the patients. The anamnestic requirements for each patient were: osteoporosis in the family, cardiovascular illness, lifestyle (dietary survey or physical activity pattern, use of smoke and alcohol).

Anthropometric measures were obtained: waist circumference (WC) was measured at the iliac crest with the patients standing; body mass index (BMI) was determined by dividing the weight (kilograms) by the square of height (meters).

For each patient was carried out a standard-12 deviation ECG, a transthoracic echocardiogram and an Eco-ColorDoppler to assess the presence of active cardiac pathologies; a liver ecography to assess the presence of liver insufficiency and steatosis was also performed.

Venous blood was withdrawn after an overnight fast. Standard laboratory techniques were adopted to assess high-density lipoprotein cholesterol (HDLc), triglycerides (TG), fasting blood glucose (FBG) and serum uric acid (SUA). Fasting insulin and PTHi concentrations were determined by enzyme-linked immune-sorbant-assay (ELISA) kit (third generation). 25(OH)D was assayed by using high-performance liquid chromatography (HPLC) and expressed in nanograms/ milliliter (ng/ml).

The insulin-resistance (IR) was estimated by using Homeostasis Model Assessment Index of Insulin Resistance (HOMA-IR). HOMA-IR was calculated with the following formula: [fasting insulin (U/ml) x fasting glucose (mmol/L) / 22.5] [21].

For the diagnosis of hypertension the clinical history and home therapy were considered. Blood pressure was measured twice to the right arm after a rest of 10 minutes with subjects in sitting position, using a mercury sphygmomanometer. Systolic (SBP) and diastolic (DBP) blood pressure levels were defined as first and fifth Korotkoff phases (mmHg).

Diabetes mellitus was defined as self-reported diabetes or by FBG ≥ 126 mg/dl.

MetS was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III criteria (NCEP ATP III), 2001, by the presence of three or more of the following features: WC ≥ 88 cm; HDLc < 50 mg/dl; FBG > 110 mg/dl or anti-diabetic treatment; fasting triglycerides ≥ 150 mg/dl; systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or antihypertensive treatment [22].

Adherence to a healthy diet was assessed using the 14-item Mediterranean diet assessment tool and obesity indexes [23]. The questions therein analyzed the number of portions and the frequent use per product of the typical Mediterranean diet (Med diet) such as olive oil, fruit, wine, fish or the intake of foods not included in the traditional Med diet. The total score ranges from 0 to 14: the higher the score, the higher the degree of adherence to the Med diet pattern. Each item was scored 0 or 1.

Physical activity habits were self-reported by patients with daily diaries. Diaries has been collected monthly.

According to the Institute of Medicine (IOM) 2011 criteria, vit. D status was categorized as follows: i) vit. D deficiency, defined by serum 25(OH)D less than 20 ng/ml; ii) vit. D insufficiency, defined by serum 25(OH) of 20-29 ng/ml; iii) vit. D sufficiency, defined by serum 25(OH)D ≥ 30 ng/ml [24].

Patients with hypovitaminosis D received an oral supplement therapy (Cholecalciferol) [25]:

1. patients with 25(OH)D < 10 ng/ml received a loading dose of 50 000 IU once a week for 4 weeks, followed by a dose of 25 000 IU once a week for 4 weeks, followed by a maintenance dose of 25 000 IU twice a month;
2. patients with 10 < 25(OH)D < 20 ng/ml received a loading dose of 25 000 IU once a week for 4 weeks, followed by a maintenance dose of 25 000 IU twice a month;
3. patients with 20 < 25(OH)D < 30 ng/ml received a loading dose of 25 000 IU twice a month for 8 weeks, followed by a maintenance dose of 25 000 IU at month;

After 6 months the subjects were re-evaluated for the

single components of MetS, IR and vit. D status.

All statistical analysis were performed using the Statistical Package for the Social Science (SPSS) version 22 for Windows. Data are presented as means ± standard deviations (sd) for continuous variables and as frequencies for discrete variables. One-way analysis of variance (ANOVA) was performed to investigate between groups differences in continuous variable while the Chi-square test ($\chi 2$) was used to explore between groups differences in discrete variable. Pearson's correlation test was used in order to investigate the relationship between variables. The Student's paired T test was used in order to compare variables' baseline values with those post vit. D supplementation therapy.

A p value below 0.05 was deemed significant.

RESULTS

Two hundred patients were enrolled (mean age: 65 ± 9.8; 45 males).

By assessing serum levels of 25(OH)D, we found that 81% of our study population had 25(OH)D < 30 ng/ml with a high prevalence of hypovitaminosis D in both sexes (83% in females *vs* 71% in males). In all, MetS was observed in 52.5%, hypertension in 65.5%, diabetes in 20.5%, nonalcoholic fatty liver in 59.5%. All diabetic subjects were on antidiabetic therapy. The style of life assessment showed poor adherence to the Med diet (88% of the sample achieved ≤ 7 points in the 14-item Med diet score, where 8, 9 o r ≥ 10 score indicates an high adherence to the Med diet) and to physical activity (only 15% of the sample reported to conduct a physical activity on a regular basis and /or 30 min brisk walk).

The relationship between vit. D levels and various conditions (hypertension, diabetes, nonalcoholic fatty liver, MetS and smoke) included in the present study was analyzed. Results showed a significant between groups difference only in MetS rate ($\chi 2$ = 13.66 p = 0.001): the presence of MetS increased with the decrease of vit. D levels. More specifically the MetS rate in the group with vit. D deficiency was higher than the rate of the groups with both vit. D insufficiency and sufficiency (respectively $\chi 2$ = 7.49 p = 0.009; $\chi 2$ = 10.32 p = 0.002).

As summarized in *Table 1*, data analysis showed a significant difference between groups in WC (p = 0.02) and PTHi (p < 0.001) values. More specifically, WC values decreased with the increase of vit. D levels: post-hoc analysis with Bonferroni correction showed a significant difference in WC values only between the groups with deficiency and insufficiency in vit. D (p = 0.04). Regarding PTHi variable, as expected, the values decreased with the increase of vit. D levels: post-hoc analysis with Bonferroni correction showed a significant difference in PTH values only between groups with deficiency and insufficiency of vit. D (p = 0.03). A between groups difference tending to the significance (p = 0.09) in both BMI and glycaemia values was observed. No further differences between groups were observed.

Analyzing the correlations between vit. D levels and the other variables included in the present study, a weak significant negative correlation was observed between vit. D levels and two components of MetS, respectively with both WC (ρ - 0.202 p = 0.004) and glycaemia values (ρ - 0.185 p = 0.009), while a negative correlation tending to the significance was noted with BMI levels (ρ - 0.135 p = 0.057). By controlling the correlation between vit. D levels and both WC, glycaemia and BMI values concurrently for the variables "gender" and "age" the significant negative correlation with WC and glycaemia values was confirmed (respectively ρ - 0.219; p = 0.002; ρ - 0.195 p = 0.005) and a significant negative correlation with the BMI values was found (ρ - 0.142 p = 0.04).

As regard the efficacy of six months oral supplementation therapy with vit. D on some of the components of the MetS tested in a group of 60 subjects with hypo-

Table 1

Groups characteristics and differences in continuous variables

Variable	Vitamin D deficiency group < 20 ng/ml (n = 109)		Vitamin D insufficiency group 20 < x < 30 ng/ml (n = 53)		Vitamin D sufficiency group > 30 ng/ml (n = 38)		F	p
	M	sd	M	sd	M	sd		
Age	65.2	10.7	64.85	8.3	62.97	9.5	0.737	n.s.
25(OH)D	12.3	4.4	24.5	2.6	43.2	15.2	247.8	0.001**
PTHi	59.23	29.7	48.8	24.3	45.8	21	4.79	0.001**
BMI	30.5	6	28.5	5.9	28.9	5.9	2.44	0.09
WC	102.7	15.5	97.6	15.4	95.6	13.7	3.92	0.02*
FBG	104.4	23.4	99	21.1	96.5	13.8	2.36	0.09
HOMA	2.9	2.25	2.8	4.5	2.3	1.7	0.616	n.s.
HDLc	56.7	17.6	59.9	16.2	55.8	14	0.882	n.s.
TG	133.9	74.7	115.8	78	122.3	50.6	1.23	n.s.
SUA	5.3	1.3	5.3	1.3	5.5	3.7	0.084	n.s.

* p < 0.05 ** p < 0 .01

25(OH)D = 25-hydroxyvitamin D; PTHi = intact parathyroid hormone; BMI = body mass index; WC = waist circumference; FBG = fasting blood glucose; HOMA = Homeostasis Model Assessment Index of Insulin Resistance; HDLc = high density lipoprotein cholesterol; TG = triglicerydes; SUA = serum uric acid.

vitaminosis D the results showed a significant increase in vit. D average levels (p = 0.001) and a significant reduction in WC values (p = 0.001) (*Table 2*).

DISCUSSION

Many studies highlight a widespread and ample diffusion of hypovitaminosis D in the world population, regardless of the seasons and latitude [26, 27]. The results of our study confirm these data and it is useful to note that all subjects examined live in Rome, that is the second sunniest city in Europe (1687 hours of sunshine/year). Furthermore, among the subjects with low levels of vit. D, 62.2% showed an important deficit, that is with values of vitamins inferior to 20 ng/ml. Another interesting datum is the high prevalence of hypovitaminosis D in both sexes. Therefore, as it is for women (whose controls are more widespread because of the major incidence of osteoporosis), also in males a proper dosage level of 25(OH)D3 is important. Furthermore, in a group substantially balanced between under/over 65 years (45% *vs* 55%), it is interesting to note that the average age of the groups examined increased while the serum levels of vit. D lessened (*Table 1*).

We found a significant association between vit. D status and MetS in line with previous reports [11, 13, 16]. Moreover, a significant negative correlation was observed between vit. D levels and two components of MetS: WC and glycaemia values. As noted, central obesity can be considered one of the main causes of the MetS [28]. The visceral fat, in fact, correlates independently with all MS components. The fatty tissue and the inflammatory alterations thereto correlated determine insulin resistance with reduced use of the marginal glucose and the alteration of the regulation and liver production of glucose. Visceral obesity and insulin resistance are furthermore associated with an increased production of adipokines, a molecule capable of causing ossidative stress, increase of inflammatory markers [tumor necrosis factors-α (TNF-α), C-reactive protein] and thrombosis as well as endothelial disfunction [29]. Normal serum vit. D may be associated with better endothelial function and can exert an active role in adipose tissue by inhibiting of chemokine and cytokine secretion in human adipocytes with benificial effects on cardiovascular health [30, 31].

We failed to find a correlation between vit. D status and serum lipid profile variables. Previous findings reported conflicting results [16, 17, 32], but differences in the study populations should be considered, as well as the failure to adjust for potential confounding variables. Levels of serum pro-inflammatory cytokines, like TNF-α and interleukin 6, are negatively correlated with serum HDL-cholesterol levels and are also associated with hypertriglyceridemia [33, 34]. Low grade chronic inflammation has been widely confirmed to be associated with MetS and vit. D has been recognized to have anti-inflammatory properties [1]. Our results suffer from a lack of knowing the inflammatory status of the patients.

Blood hypertension was not found to be associated with 25(OH)D. Pre-existing data are mixed [35-37] and confounded by differences in the study populations. Our results could be influenced by intra-groups differences (years since menopause for women, inflammatory status, different cardiovascular risk etc.). Future investigation on this topic are welcome.

The identification and treatment of hypovitaminosis D could be important not only in youth but also in elderly, in which MetS and in particular abdominal obesity are potential risk factors for disability [38]. It is apparent, therefore, the need to maintain sufficient plasmatic levels of vit. D; however, there is no agreement on how to attain this objective [39, 40]. In this study, six months of vit. D supplementation in 60 subjects has been proven to be efficacious in restoring normal levels of 25(OH)D3 in most of the patients, without side effects. Furthermore, after the supplementation therapy, a significant reduction ($p < 0.01$) of the WC values was observed. It is common knowledge that overweight subjects with increased WC are exposed to an higher risk of type 2 diabetes, hypertension and stroke [41]. In our samples we found a significant reduction of the WC after supplementation therapy which confirms an important role of the vit. D in the reduction of cardiovascular risk, also in consideration of the low adherence to the Med diet and to physical activity. In this respect, a recent study in young patients with BMI superior to 30 – regardless of the diet followed and of other possible variables which may interfere – has shown high concentrated levels of calcium plus vit. D, correlated with a major body fat and visceral fat loss after three months [42]. Recently, nu-

Table 2
Effect of 6 months oral supplementation therapy in 60 subjects with hypovitaminosis D

Variable	Pre vitamin D oral supplementation therapy		Post vitamin D oral supplementation therapy		t	p
	M	sd	M	sd		
25(OH)D	15.3	6.9	33.3	14.9	-9.19	0.001*
WC	99.2	16.3	95.9	15.7	4.33	0.001*
FBG	100.3	22.8	97.1	16.7	1.01	n.s.
HDLc	56.5	16.4	58.4	15.8	-0.752	n.s.
TG	127.6	58.3	115.8	54.1	1.25	n.s.

*p < 0.01
25(OH)D = 25-hydroxyvitamin D; WC = waist circumference; FBG = fasting blood glucose; HDLc = high density lipoprotein cholesterol; TG = triglicerydes; SUA = serum uric acid.

merous evidences have been provided that obesity can also be the consequence of an increased PTH levels and low vit. D status. Increased PTH levels enhances lipogenesis by an increased calcium influx into the adipocytes [43]. Calcium and vit. D are important determinants of PTH levels, and several studies have shown that the supplementation of these nutrients resulted also in weight loss [42-44].

Nevertheless the current study faces certain limitations. Firstly, this study was based on a single measurement of serum 25-(OH) vit. D as an indicator of vit. D status. Secondly, it is a cross-sectional study with small sample size. Thirdly, although the Authors adjusted for many potential confounders, residual confounding may remain (*e.g.* time spent outdoors, etc.).

Further studies with larger samples should be carried out to confirm the correlation between hypovitaminosis D and MetS, in order to evaluate the effects of a supplementation therapy on cardiovascular risk.

CONCLUSIONS

The results of our study demonstrate that:

- hypovitaminosis D is a condition quite common in both sexes;
- there is a significant negative correlation between levels of vit. D and MetS, along the lines demonstrated by other studies. Furthermore, vit. D levels are correlated with two MetS components: glycaemia and WC;
- after supplementation therapy in subjects with hypovitaminosis D a significant WC reduction was observed, even with a low adherence to the healthy diet and to the physical activity;
- we believe it is indispensable to measure out the levels of vit. D – especially in subjects over 45 years old – and, when necessary, to provide an adequate supplementation therapy in order to aid the positive pleiotropic effects that this vitamin exerts also in reducing cardiovascular risks.

Conflict of interest statement

None.

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