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RESEARCH ARTICLE

25 Hydroxyvitamin D Levels are Negatively and Independently Associated with Fat Mass in a Cohort of Healthy Overweight and Obese Subjects

Giovanni De Pergola[1,*], Tommaso Martino[2], Roberta Zupo[1], Domenico Caccavo[1], Claudio Pecorella[1], Silvia Paradiso[1], Franco Silvestris[1] and Vincenzo Triggiani[3]

[1]*Department of Internal Medicine and Clinical Oncology, Clinical Nutrition Unit, Medical Oncology, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy;* [2]*Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare 11, 70124 Bari, Italy;* [3]*Interdisciplinary Department of Medicine, Section of Internal Medicine, Geriatrics, Endocrinology and Rare Diseases, University of Bari, School of Medicine, Policlinico, Piazza Giulio Cesare, 70124 Bari, Italy*

Abstract: ***Background:*** Obesity is associated with lower serum vitamin D (25(OH)D) levels through several mechanisms. The aim of the study was to examine the possibility of a negative association between fat mass and 25(OH)D levels in a cohort of otherwise healthy overweight and obese subjects, independently of age, sex, blood pressure levels and anthropometric and metabolic parameters

Materials and Methods: 147 overweight and obese subjects (106 women and 41 men), aged between 18 and 69 years, were enrolled into the study. All of them did not show any clinically evident metabolic or chronic diseases (*i.e*. hypertension, diabetes mellitus, renal failure, *etc*.) and did not use any kind of drug. Serum fasting levels of 25(OH)D, insulin, glucose, uric acid and lipids (triglycerides, total, HDL and LDL cholesterol) were measured. The season in which the blood samples were collected was autumn. Insulin resistance was assessed by using the Homeostasis Model Assessment (HOMA-IR). Body composition parameters (Fat Mass [FM], Fat Free Mass [FFM], body cell mass [BCM], Total Body Water [TBW]) were measured by electrical Bioimpedance Analysis (BIA). Lastly, demographic, anthropometric and clinical parameters (age, Body Mass Index [BMI], Waist Circumference [WC], Systolic (SBP) and Diastolic (DBP) blood pressure) were also assessed.

Results: 25(OH)D levels were significantly and negatively correlated with BMI ($P < 0.001$), WC ($P < 0.01$), DBP ($P < 0.05$), insulin ($P < 0.001$), HOMA-IR ($P < 0.01$), triglycerides ($P < 0.01$), and fat mass ($P < 0.001$). A multivariate regression analysis was performed by considering 25(OH)D levels as the dependent variable and sex, waist circumference, fat mass, DBP, triglycerides, and insulin (or HOMA-IR) as the independent ones, and 25(OH)D levels maintained a significant and independent relationship only with fat mass (negative) ($P < 0.01$).

Conclusion: This study clearly shows that 25(OH)D circulating levels are progressively lower with the increase of fat mass, independently of sex, body fat distribution, blood pressure and insulin and metabolic parameters. These data strongly show that adipose tissue accumulation per se is absolutely the main factor responsible factor for lower 25(OH)D levels in obese subjects, possibly through sequestration of fat soluble 25(OH)D in fat mass.

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1. INTRODUCTION

25-hydroxyvitamin D (25(OH)D, or calcidiol) is the major circulating and storage form of 25(OH)D (1,2), and evaluation of serum 25(OH)D is considered to provide a reliable parameter of the vitamin D status (1,2). Vitamin D exerts its canonical roles on the musculo-skeletal system and in the calcium/phosphorus homeostasis. However, in the last years, increasing evidences indicated that vitamin D may produce several extra-skeletal effects, which may contribute to the pathogenesis of several non-skeletal disorders [1, 2]. In fact, vitamin D seems to influence insulin secretion and sensitivity, and low vitamin D levels have been shown to predict type 2 diabetes [3, 4]. Vitamin D supplementation has been reported to reduce blood pressure levels in normal subjects [5] and this vitamin has been suggested to protect against cardiovascular risk and mortality [6]. Moreover, vitamin D deficiency may interfere with the occurrence of inflammation [7], cancer [7] and several autoimmune diseases [8, 9]. Lastly, vitamin D may influence male and female fertility [10], bowel function [11] and the onset of neurological diseases [12].

*Address correspondence to this author at the Via Putignani 236 - 70122 Bari, Italy; Tel; +39-080-5592909; Fax: +39-080-5478831;
E-mail: giovanni.depergola@uniba.it

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Obesity is characterized by lower 25(OH)D levels through several potential mechanisms [3, 13, 14]: the most important is commonly considered the sequestration of fat soluble vitamin D in higher fat mass, but this is still an assumption and not a proven explanation.

Accordingly, weight loss is associated with an increase in serum 25-hydroxyvitamin D in overweight or obese women [15, 16], even though an impaired release of vitamin D has been demonstrated in dysfunctional adipose tissue (AT), as AT present in obesity [17].

On the other hand, there is evidence that vitamin D may be a predictor of resting metabolic rate [18] and regulate AT differentiation, metabolism and mass [13, 14], thus inhibiting the risk of obesity. Therefore, it may well be that vitamin D and AT may influence each other.

To date, systematic reviews and meta-analyses that investigated the relationship between 25(OH)D and fat mass have given inconsistent findings [19], and several randomized clinical trials (RCTs) investigating the influence of cholecalciferol supplement on the percentage of fat mass have given conflicting results [19].

Actually, no study has been specifically performed to investigate the association between vitamin D levels and all the parameters of body composition in obesity, independently of age, sex, anthropometric parameters, blood pressure levels, and insulin and metabolic parameters in healthy overweight and obese subjects.

The aim of the current study was just to investigate this possibility in a population (147 subjects) of overweight and obese subjects not taking any kind of drug. We assessed the relationship between 25(OH)D levels and body mass index (BMI), waist circumference (waist), blood pressure levels, and fasting serum insulin, uric acid, glucose, and lipid (triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol) concentrations. Body composition parameters were measured by Bioimpedance (BIA) (Fat Mass [FM] Fat Free Mass [FFM], Body Cell Mass [BCM], and Total Body Water [TBW]).

2. MATERIALS AND METHODS

2.1. Subjects

Subjects were recruited consecutively at the Outpatient Clinic of Nutrition of the Medical Oncology Unit, Department of Internal Medicine and Clinical Oncology, University of Bari, School of Medicine, Policlinico, Bari, Italy. The subjects were referred to the Outpatient Clinic with the aim of improving the quality of their diet and/or to loose body weight. They were enrolled at the first visit whether they showed a Body Mass Index (BMI)) > 25.0 kg/m^2 and did not take any kind of drugs (including oral contraceptives or drugs for osteoporosis). Exclusion criteria concerned subjects suffering from endocrine diseases (diabetes mellitus, hypo or hyperthyroidism, hypopituitarism *etc.*), unstable hypertension, chronic inflammatory diseases, renal and liver failure, angina pectoris, myocardial infarction, heart failure, genetic heart diseases, minor and major stroke.

The group of participants was made up of 147 overweight and obese subjects (106 women and 41 men), aged between 18 and 69 years. Patients were defined overweight if they had a BMI between 25.0 and 29.9, and obese if they had a BMI $\geq$ 30.

Subjects were examined by means of medical history, routine tests and electrocardiogram (ECG). None was following a rigid low-calorie diet or carrying out a high level of physical activity, while each of them kept on doing their common lifestyle.

2.2. Clinical and Biochemical Assessment

Body weight was rounded to the nearest kg. Height was determined to the nearest cm. BMI was calculated as body weight (kg)/ height $(m)_2$. The waist circumference was measured at the narrowest part of the abdomen, or in the area between the 10th rib and the iliac crest (minimum circumference). The blood pressure of ambulatory patients was determined in a sitting position after at least 10-min rest and at least three different times, using a mercury manometer with appropriately sized cuff. Blood samples were drawn between 08:00 and 09:00 a.m. after an overnight fast. The season in which the blood samples were collected was autumn (from 22 september to 21 December 2017).

Serum 25(OH)D was quantified by a chemiluminescence method (Diasorin Inc, Stillwater, USA), and all samples were analyzed in duplicate [3]. Serum insulin concentrations were measured by radioimmunoassay (Behring, Scoppito, Italy). Plasma glucose was determined using the glucose oxidase method (Sclavus, Siena, Italy), while the concentrations of plasma lipids (triglycerides, total cholesterol, HDL cholesterol) were quantified by automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald equation [20]. The concentrations of serum UA were determined by the uricase/POD method implemented in an autoanalyzer (Boehringer Mannheim, Mannheim, Germany).

2.3. Definition of Insulin Resistance

Insulin resistance was determined through the use of homeostasis model assessment (HOMA-IRIR) [21].

2.4. Body Composition Parameters

Subjects underwent body composition evaluation. Body composition parameters (fat mass [FM], fat free mass [FFM], body cell mass [BCM], total body water [TBW]) were measured by electrical bioimpedance analysis (BIA). In practice, a 50 kHz, tetra-polar phase-sensitive BIA (BIA-101 ASE; AKERN-Srl, Florence, Italy) injecting a sinusoidal alternating current of 400 μA_{RMS} was used to measure resistance to the nearest 1.0 Ω (R), reactance to the nearest 0.1 Ω (Xc) and phase angle to the nearest 0.1º (PA).

2.5. Statistical Analysis

The sample characteristics were expressed as mean $\pm$ Standard Deviation (SD) unless otherwise indicated. Shapiro-Wilk test was used to determine whether the variables showed a normal distribution. The Student's *t*-test (*t*) was used to compare continuous variables and the *chi-*

squared test was applied to compare categorical variables (*e.g*. sex), as appropriate.

P-values less than 0.05 were considered statistically significant. Variables with *p*-value lower than 0.05 in the univariate analysis were included in a multivariate linear regression model finalized to evaluate parameters independently related to 25(OH)D levels. Statistical analysis was performed using the computing environment R version 3.3.1 (R Development Core Team, 2016).

3. RESULTS

Table **1** sums up the general, anthropometric, hormone and metabolic parameters and the BIA parameters of the whole population enrolled in the study, and the same parameters stratified by sex. Waist circumference, systolic (SBP) and diastolic blood pressure (DBP), insulin, HOMA-IR, triglycerides (TG), uric acid, FFM, BCM and TBW were significantly higher in men than in women. HDL-cholesterol was higher in women than in men. 25(OH)D, age, BMI, fat mass, blood glucose, total and LDL-cholesterol were not significantly different with sex.

Table **2** shows the univariate relationships between 25(OH)D levels and all other parameters. 25(OH)D showed a significant negative association with fat mass, BMI, waist circumference, DBP, insulin, HOMA-IR, and TG. 25(OH)D did not show a significant relationship with FFM, BCM, TBW, age, SBP, uric acid, total, LDL- and HDL-cholesterol.

Table **3** shows the results of a multivariate analysis, performed with 25(OH)D levels as the dependent variable and DBP, TG, fat mass, insulin and sex as the independent ones (**Model 1**). Fat mass (negatively) and sex were the only parameters to maintain a significant and independent relationship with 25(OH)D levels. Results did not change when HOMA-IR entered into the regression instead of insulin levels. Another multivariate analysis was performed introducing also waist circumference among independent variables (**Model 2**). In this case, fat mass (negatively) was the only parameter to maintain a significant and independent relationship with 25(OH)D levels (Fig. **1**). Results did not change when HOMA-IR entered into the regression instead of insulin levels.

4. DISCUSSION

This study confirms that obesity is associated with lower serum vitamin D (25(OH)D) concentrations and it shows a strong negative relationship between 25(OH)D levels and fat mass, independently of age, sex, body fat distribution, blood pressure levels, and insulin and metabolic parameters (fasting blood glucose, lipids, and uric acid) in healthy

Table 1. General, anthropometric, hormone, metabolic and BIA parameters (n=147 subjects).

Variable	Whole Population Mean (SD)	Male Mean (SD)	Female Mean (SD)	P
Age (years)	42.31 (12.42)	41.95 (12.82)	42.45 (12.32)	0.832
BMI (Kg/m^2)	33.19 (5.53)	33.82 (6.40)	32.94 (5.16)	0.437
Waist circumference (cm)	107.94 (12.71)	115.00 (14.31)	105.21 (10.94)	<0.001
Systolic blood pressure (mmHg)	128.17 (14.71)	134.88 (12.66)	125.57 (14.68)	<0.001
Diastolic blood pressure (mmHg)	82.31 (11.00)	88.41 (10.79)	79.94 (10.16)	<0.001
25(OH)D (ng/ml)	20.58 (7.63)	22.03 (8.37)	20.02 (7.28)	0.181
Blood glucose (mg/dl)	88.97 (11.25)	91.29 (11.10)	88.07 (11.23)	0.120
Insulin(μU/ml)	13.08 (8.79)	16.61 (10.89)	11.72 (7.45)	0.010
HOMA-IR	2.90 (2.06)	3.78 (2.49)	2.56 (1.76)	0.005
Triglycerides (mg/dl)	101.89 (52.82)	128.61 (59.66)	91.47 (46.15)	<0.001
Total cholesterol (mg/dl)	197.09 (39.83)	188.46 (36.53)	200.43 (40.71)	0.088
LDL-cholesterol (mg/dl)	124.15 (34.91)	121.98 (34.93)	124.99 (35.03)	0.640
HDL-cholesterol (mg/dl)	53.35 (14.71)	44.09 (11.82)	56.92 (14.19)	<0.001
Uric acid (mg/dl)	4.53(1.52)	5.75 (1.64)	4.04 (1.15)	<0.001
Fat mass (Kg)	34.95 (11.97)	33.83 (14.04)	35.39 (11.11)	0.526
Fat-free mass (Kg)	55.78 (12.05)	70.54 (10.03)	50.07 (6.76)	<0.001
Body cell mass (Kg)	30.96 (7.44)	40.54 (5.98)	27.25 (3.71)	<0.001
Total Body Water (Lt)	41.22 (8.46)	51.92 (7.35)	37.05 (4.03)	<0.001

Table 2. Relationship between 25(OH)D levels and all parameters.

Variable	r	*P*
Age (years)	-0.001	ns
BMI (Kg/m2)	-0.303	< 0.001
Waist circumference (cm)	-0.112	< 0.01
Systolic BP (mmHg)	-0.048	ns
Diastolic BP (mmHg)	-0.104	< 0.05
Fat mass (Kg)	-0.188	< 0.001
Fat-free mass (Kg)	-0.009	ns
Body cell mass (Kg)	-0.018	ns
Total body water (Lt)	-0.020	ns
Blood glucose (mg/dl)	-0.058	ns
Insulin (μU/ml)	-0.158	< 0.01
HOMA-IR-IR	-0.148	< 0.01
Triglycerides (mg/dl)	-0.152	< 0.01
Total cholesterol (mg/dl)	-0.017	ns
LDL-cholesterol	-0.013	ns
HDL-cholesterol	-0.015	ns
Uric acid (mg/dL)	-0.037	ns

Table 3. Multiple regression analysis performed in all subjects (n=147), by considering.

Independent Variables	Estimate	Std. Error	T	P
Model 1				
(Intercept)	34.130	4.828	7.069	< 0.001
Diastolic blood pressure	-0.073	0.058	-1.255	Ns
Triglycerides	-0.019	0.012	-1.557	Ns
Fat mass	-0.159	0.055	-2.879	< 0.01
Insulin	-0.060	0.078	-0.776	Ns
Sex	3.248	1.495	2.173	<0.05
Model 2				
Intercept	22.997	7.934	2.899	<0.01
Waist circumference	0.160	0.091	1.761	Ns
Diastolic blood pressure	-0.085	0.058	-1.457	Ns
Triglycerides	-0.022	0.012	-1.794	Ns
Fat mass	-0.285	0.090	-3.160	<0.01
Insulin	-0.068	0.078	-0.880	Ns
Sex	1.745	1.712	1.019	Ns

25(OH)D levels as the dependent variable and diastolic blood pressure (BP), triglycerides, fat mass, insulin and sex as independent variables (Model 1), or waist circumference, diastolic blood pressure (BP), triglycerides, fat mass, insulin and sex as independent variables (Model 2).

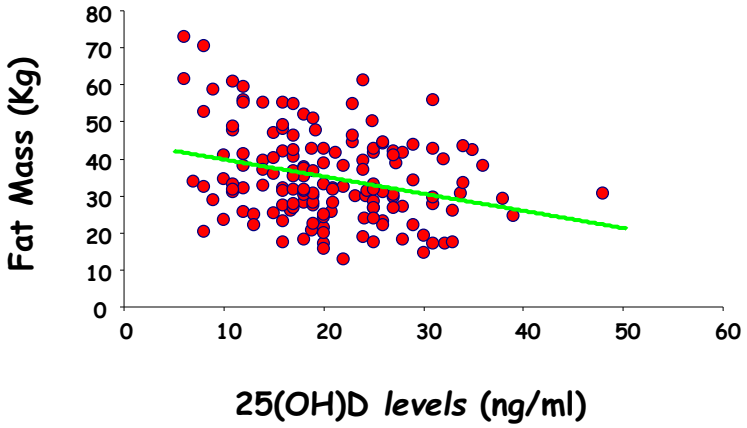

Fig. (1). Shows the negative correlation between serum levels of vit. D and fat mass.

overweight and obese subjects, not treated with any kind of drugs.

Since vitamin D has several protective effects on the health status, it may reduce the risk of hypertension, cardiovascular diseases, type 2 diabetes, infertility, autoimmune diseases, cancer, and bowel and neurological diseases [3-12], the progressive decrease of vitamin D levels with a parallel increase of body fat accumulation may partly explain several negative effects of obesity.

At variance with body fat, we could not find any correlation between 25(OH)D levels and fat free mass and its components BCM and TBW. Therefore, even though some multicenter study demonstrated that post-menopausal women with vitamin D deficiency had a significant reduction of appendicular muscle strength and physical performance [22], the effect of body fat in decreasing 25(OH)D levels seems to be stronger than the possible effect of vitamin D on FFM, BCM and TBW.

Concerning the effect of vitamin D on body fat, the randomized clinical trials (RCTs) that have studied the influence of cholecalciferol supplement on percentage fat mass have given conflicting results [19], thus suggesting that the effect of vitamin D on body fat is much weaker than the effect of body fat on 25(OH)D levels. We believe that supplementation with 25-hydroxyvitamin-D3 is effective in obese individuals, and should be proposed in these patients. The recent demonstration that dysfunctional adipose tissue, as in obese patients, shows a reduced catecholamine-induced release of 25-hydroxyvitamin-D3 and altered activity of vitamin D–metabolizing enzymes [17] is in line with our suggestion.

We found a significant negative association between insulin and 25(OH)D levels. This may suggest that insulin resistance and secondary hyperinsulinemia has a role in lowering 25(OH)D levels in overweight and obese patients, as previously suggested just by our group [3]. However, in the present study, 25(OH)D levels did not maintain a significant relationship with insulinemia when a multiple regression was performed by considering 25(OH)D levels as the dependent variable and sex and fat mass were entered into the statistical analysis. It may well-known be that low 25(OH)D may be responsible for insulin resistance, but this hypothesis is still questionable. Cefalo CMA et al have shown that cholecalciferol supplementation, combined with a weight loss program, significantly improves insulin sensitivity in healthy subjects with obesity [23]. Mousa A et al have shown that vitamin D supplementation has no effect on insulin sensitivity or secretion in vitamin D-deficient, overweight or obese adults [24].

Apart from anthropometric and body composition parameters, insulin levels and insulin resistance, vitamin D showed a significant negative association with triglycerides and DBP. The negative correlation between 25(OH)D and triglyceride is well known [3] and possibly reflects the associated direct influence of obesity, hyperinsulinemia and decreasing levels of 25(OH)D [25] on TG levels. The inverse association between vitamin D and DBP possibly reflects the favourable effect of vitamin D on blood pressure [26], even though we cannot explain why 25(OH)D levels were significantly associated with diastolic, but not with systolic blood pressure.

CONCLUSION

The present study shows a negative association between 25(OH)D levels and body fat independently of age, gender, body fat distribution, insulin, metabolic parameters and blood pressure in subjects with uncomplicated obesity. Since vitamin D has several protective effects on health, obesity may partly exert its negative effects through the decrease of vitamin circulating levels.

Therefore, since obese patients are individuals at risk for vitamin D deficiency, they should be screened by the 25-hydroxy-vitamin D assay, and preferentially supplemented with calcifediol because of an altered activity of vitamin D–metabolizing enzymes [17, 27].

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

All participants signed an informed consent form.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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