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Vitamin D deficiency is associated with insulin resistance independent of intracellular calcium, dietary calcium and serum levels of parathormone, calcitriol and calcium in premenopausal women

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Abstract

Background: **There is evidence that vitamin D deficiency is associated with increased risk of cardiovascular disease. However, it is not known if this association is independent of dietary calcium, intracellular calcium and serum levels of parathormone, calcitriol and calcium.**

Objectives: **To investigate the independent relationship of vitamin D deficiency with insulin resistance, lipid profile, inflammatory status, blood pressure and endothelial function.**

Method: **Cross-sectional study conducted with 73 healthy Brazilian premenopausal women aged 18 – 50 years. All participants were evaluated for: 25 hydroxyvitamin D serum levels, anthropometric parameters, body composition, calcium metabolism, insulin resistance, lipoprotein profile, inflammatory status, blood pressure and endothelial function. Endothelial function was assessed by reactive hyperemia index using Endo-PAT 2000®. Women were stratified in two groups: with vitamin D deficiency (25 hydroxyvitamin D < 20 ng/ml; n=12) and without vitamin D deficiency (25 hydroxyvitamin D ≥ 20 ng/ml; n=61).**

Results and discussion: **Participants with vitamin D deficiency compared with those without deficiency of this vitamin had significantly higher levels of glucose (88.25 ± 3.24 vs. 80.15 ± 1.13 mg/dl), greater HOMA-IR (6.43 ± 0.73 vs. 4.42 ± 0.25) and lower reactive hyperemia index (1.68 ± 0.1 vs. 2.17 ± 0.1). After adjustments for confounding factors including age, body mass index, waist circumference, dietary calcium, intracellular calcium and serum levels of parathormone, calcitriol and calcium differences between groups remained significant, regarding glucose and HOMA-IR.**

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LA DEFICIENCIA DE VITAMINA D SE ASOCIA CON RESISTENCIA A LA INSULINA INDEPENDIENTE DEL CALCIO INTRACELULAR, EL CALCIO DE LA DIETA Y LOS NIVELES SÉRICOS DE HORMONA PARATIROIDEA, CALCITRIOL Y CALCIO EN MUJERES PREMENOPÁUSICAS

Resumen

Introducción: **Hay evidencias de que la deficiencia de vitamina D se asocia con mayor riesgo de enfermedad cardiovascular. Sin embargo, no se sabe si esta asociación es independiente de calcio en la dieta, el calcio intracelular y los niveles séricos de hormona paratiroidea, calcitriol y calcio.**

Objetivos: **investigar la relación independiente de la deficiencia de vitamina D con resistencia a la insulina, el perfil lipídico, el estado inflamatorio, la presión arterial y la función endotelial.**

Métodos: **Estudio transversal realizado con 73 mujeres pre menopáusicas sanas brasileñas con edad 18-50 años. Todos los participantes fueron evaluados para: niveles séricos de 25 hidroxivitamina D, parámetros antropométricos, la composición corporal, metabolismo del calcio, resistencia a la insulina, el perfil de lipoproteínas, estado inflamatorio, la presión arterial y la función endotelial. La función endotelial fue evaluada por el índice de hiperemia reactiva mediante el uso de Endo-PAT 2000®. Las mujeres fueron estratificados en dos grupos: con deficiencia de vitamina D (25 hidroxivitamina D <20 ng / ml; n = 12) y sin deficiencia de vitamina D (25 hidroxivitamina D ≥ 20 ng / ml; n = 61).**

Resultados y Discusión: **Los participantes con deficiencia de vitamina D en comparación con aquellos sin deficiencia de esta vitamina tenían niveles significativamente más altos de glucosa (88.25 ± 3.24 vs. 80.15 ± 1.13 mg/dl), mayor índice HOMA-IR (6.43 ± 0.73 vs. 4.42 ± 0.25) y menor índice de hiperemia reactiva (1.68 ± 0.1 vs. 2.17 ± 0.1).Después de los ajustes por factores de confusión como la edad, índice de masa corporal, circunferencia de la cintura, el calcio en la dieta, el calcio intracelular y los niveles séricos de hormona paratiroidea, calcitriol y calciolas diferencias entre los grupos siguieron siendo significativas, con respecto a la glucosa y el HOMA-IR.**

Conclusions: **The findings of the present study suggest that vitamin D deficiency is associated with insulin resistance independent of dietary calcium, intracellular calcium and serum levels of parathormone, calcitriol and calcium in healthy premenopausal women.**

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Key words: *Vitamin D. Insulin resistance. Endothelial function. Glucose. Parathormone.*

Conclusiones: **Los resultados del presente estudio sugieren que la deficiencia de vitamina D se asocia con resistencia a la insulina independiente de calcio en la dieta, el calcio intracelular y los niveles séricos de hormona paratiroidea, calcitriol y calcio en mujeres pre menopáusicas sanas.**

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Palabras clave: *Vitamina D. Resistencia a la insulina. La función endotelial. Glucosa. La hormona paratiroidea.*

Introduction

Epidemiologic studies have linked vitamin D deficiency with increased risk of major cardiovascular (CV) events, CV mortality and all-cause mortality[1-3]. The relationship between vitamin D deficiency and some CV risk factors such as insulin resistance, type 2 diabetes, hypertension and endothelial dysfunction, has also been pointed out in some observational studies[4-7], but not in others[8-11].

Vitamin D deficiency decreases intestinal calcium absorption which reduces serum calcium and triggers parathormone (PTH) release in order to correct serum calcium. 25 hydroxyvitamin D [25(OH)D] levels are inversely associated with PTH levels. The threshold for PTH elevation is 25(OH)D level of 30 ng/ml and further reduction will result in higher PTH levels. Secondary hyperparathyroidism caused by vitamin D deficiency may mediate many of the detrimental effects of inadequate 25(OH)D levels[12]. In fact, higher levels of PTH have been associated with different CV risk factors[13-16]. Reis et al observed that elevated PTH levels were associated with an increased prevalence of metabolic syndrome, but failed to find association between vitamin D deficiency and metabolic syndrome[10]. Soares et al suggested an inverse relationship between PTH and insulin sensitivity, independent of vitamin D[16].

Based on the complex interrelation of 25(OH) D with PTH and serum calcium, and on the possible association of PTH and serum calcium with CV risk, some studies that evaluated the association of vitamin D deficiency with increased CV risk made statistical adjustments for PTH and/or serum calcium[3,7,17].

Calcium intake may also interfere in PTH levels. A low dietary calcium intake reduces serum calcium and stimulates the release of PTH[18]. There are evidence that reduced dietary calcium intake is associated with higher prevalence of obesity, metabolic syndrome, type 2 diabetes and hypertension; CV events; and all-cause or CV mortality[19]. Forrest and Stuhldreher (2011) observed that vitamin D deficiency is significantly more common among those not consuming milk daily[20] and some studies evaluated the association of both serum 25(OH)D and dietary calcium with CV risk factors[21,22].

One of the proposed mechanisms that explains the relationship of PTH with CV risk is that PTH activates renal conversion of 25(OH)D into 1,25(OH)2D, increasing serum levels of this active metabolite, which can increases intracellular calcium concentration in different cell types including adipocytes and vascular smooth muscle cells. Recent evidence have suggested that increased intracellular calcium concentration may be associated with CV risk factors including obesity, insulin resistance and increased blood pressure[18,19].

To our knowledge, the previous studies linking low 25(OH)D with an increased CV risk did not simultaneously controlled their results for factors that are interrelated with 25(OH)D and that may also interfere in CV risk such as dietary calcium, intracellular calcium and serum levels of PTH, calcitriol and calcium.

Thus, the aim of the present study was to investigate in a sample of healthy Brazilian premenopausal women the independent relationship between vitamin D deficiency and insulin resistance, lipoproteins profile, inflammatory status, blood pressure and endothelial function.

Materials and methods

This cross-sectional study was carried out at the Laboratory of Clinical and Experimental Pathophysiology - CLINEX, located at Pedro Ernesto University Hospital of Rio de Janeiro State University.

Participants were selected at the Department of Plastic Surgery among candidates for lipoplasty and at the Department of Gynecology among participants in the family planning program. Inclusion criteria were women aged between 18 and 50 years without menopausal status. Exclusion criteria were: current use of calcium and vitamin D supplementation, as well as use of medications that could interfere with calcium and vitamin D metabolism, regular physical activity (at least 30 minutes/day on at least 3 days/week), use of drugs for weight loss, and use of antihypertensive, antidiabetic and lipid-lowering drugs. Exclusion criteria also included the following conditions: changes in body weight (>3 kg) within previous 6 months; smoking; eating disorders; major depression; any metabolic disease, such as diabetes mellitus or hypothyroidism; any chronic diseases severely affecting the CV, gastrointestinal, and renal systems; and pregnancy or lactation.

Women who met eligibility criteria and agreed to participate were included into the study. Clinical, an-

thropometric, biochemical and endothelial function evaluations were performed after 12h fast between 8 to 10 a.m.

Nutritional Assessment

Anthropometric measurements were taken twice and mean values were used in all analyses. Participants were wearing light clothing and with no shoes during the measurements of weight and height. A calibrated scale accurate to ± 0.1kg was used to determine body weight; height was measured using a stadiometer accurate to ± 0,5cm (Filizola S.A., São Paulo, SP, Brazil). Body mass index (BMI) was calculated using the standard equation (kg/m^2)[23] and was used to evaluate total body adiposity (excessive adipose tissue, independent of site).

To evaluate abdominal adiposity, waist circumference (WC), waist-to-rip ratio, and waist-to-height ratio were used. An inextensible measuring tape was used to measure WC and hip circumference with the participants in the standing position. WC was determined midway between the lower margin of the last rib and the iliac crest. The measurements were taken at mid-exhalation[23]. Hip circumference was measured at the widest point of the hip/buttocks area with the measuring tape parallel to the floor. Waist-to-rip ratio was calculated by dividing WC (cm) by hip circumference (cm), and the ratio between WC (cm) and height (cm) defined waist-to-height ratio.

Percentage of body fat was estimated by electrical bioimpedance using Biodynamics BIA-450 body fat analyser (Biodynamics Corp., Seatle, WA, USA). Patients were in supine position with arms and legs lying parallel and separated, so that the thighs were not touching. Two electrodes were placed on the hand and wrist, and two others were positioned on the foot and ankle of the right side of the body. Resistance and reactance were measured and the software provided by the manufacturer calculated percentage of body fat.Values of percentage of body fat were also used to evaluate total adiposity.

A semi-quantitative food frequency questionnaire was used to assess the usual dietary intake of calcium, energy, proteins, carbohydrates and lipids over the previous 6 months. This food frequency questionnaire, containing eighty items and usual portions, was developed for the Brazilian population based on commonly consumed foods and was validated against more accurate methods of dietary intake assessment[24].

Laboratory parameters

Blood samples were collected after a 12-hour fasting period and processed considering the specifications of each biochemical variable. Aliquots of plasma and serum were stored at -20°C or -80°C for future analysis. Laboratory parameters included fasting circulating levels of creatinine, calcium, 25(OH)D, $1,25(OH)_2D$, PTH, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, leptin, high-sensitivity C-reactive protein (hs-CRP) and adiponectin. Participants were asked to collect 24 h urine and urinalysis was performed for calcium and creatinine.

To evaluate vitamin D status, levels of 25(OH)D were determined by radioimmunoassay Double Antibody method using commercial kit (DiaSorin SpA, Saluggia, Vercelli, Italy). Vitamin D deficiency was defined by serum 25(OH)D below 20 ng/ml[28]. Plasma PTH (intact molecule) and 1,25(OH)2D were determined by ELISA using commercially available kits (Uscn Life Science Inc., Missouri, USA). Serum and urinary calcium was determined by colorimetry. In erythrocytes, intracellular calcium concentration was measured by atomic absorption spectrometry, using the modified method of Cheng et al[25]. Serum and urinary creatinine was assessed by kinetic method. Ionized serum calcium was calculated using serum proteins values determined by colorimetry.

Fasting plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin levels were determined by radioimmunoassay method using the commercially available human insulin specific kit (EMD Millipore Corporation, Billerica, MA, USA). Homeostasis model assessment of insulin resistance index (HOMA-IR) was used to assess insulin resistance status, calculated by diving the product fasting insulin (μU/ml) x fasting plasma glucose (mmol/l) / 22.5.

Total cholesterol, HDL-cholesterol and triacylglycerol concentrations were assessed by an automated analyzer (Du Pont Co., Wilmington, DE, USA). LDL-cholesterol was estimated using Friedewald's formula when triacylglycerol values were lower than 400 mg/dl. Leptin was determined by radioimmunoassay using a commercially available kit (Linco Research Inc., Missouri, USA).

To evaluate biomarkers of inflammatory state, circulating levels of adiponectin and hs-CRP were determined. The multiplex method was used to assess serum adiponectin (EMD Millipore Corporation) and turbidimetry was used to determine hs-CRP (Helica Biosystems, Inc.).

Blood Pressure

Blood pressure and heart rate were recorded using a calibrated Dinamap 1846 Critikon automated sphygmomanometer (Critikon, Tampa, FL, USA). These variables were measured six times, the first value was discarded, and the mean of the last five readings was used. Participants remained resting for at least 10 min in the sitting position before the measurements. An appropriate arm cuff was used on the non-dominant arm. Arm position was adjusted so that the cuff was at the level of the right atrium.

Endothelial function

Endothelial function was evaluated by peripheral arterial tonometry method, using Endo-PAT 2000®, a finger plethysmographic device (Itamar Medical Ltd, Caesarea, Israel). This non-invasive method offers the possibility of an easy and rapid assessment of vascular function in which data are analysed independently of the examiner[26]. Alterations in pulsatile arterial volume detected by peripheral arterial tonometry have been associated with flow-mediated dilatation measurement result[27]. The measurements were performed through fingertip probes placed on both index fingers and pulse wave amplitudes were detected and recorded. A five-minute measurement was taken at baseline. Sequentially, arterial flow was occluded using a cuff on the non-dominant arm which was inflated. The cuff was rapidly deflated after 5 minutes of occlusion to allow reactive hyperemia. The following 5 minutes were also recorded. The other arm served as a control and the difference between the two arms was used by Endo-PAT 2000® software to automatically calculate the reactive hyperemia index.

Statistical methods

Based on 25(OH)D serum levels, participants were stratified into two groups: the first group was composed of participants with 25(OH)D concentration < 20 ng/ml (vitamin D deficiency group); and the other group was composed of participants with 25(OH)D concentration ≥20 ng/ml (without vitamin D deficiency group).

Means ± standard errors were used to describe continuous variables which were compared between groups using unpaired Student's t-test. Multiple linear regression was used to adjust for confounding factors. Categorical variables were summarized as absolute number and relative frequency. Chi-square test was used for comparisons among proportions. Normality was tested by using the Shapiro-Wilk normality test. Skewed data (age, body weight, BMI, WC, hip circumference, Waist-to-height ratio, systolic blood pressure, heart rate, glucose, triacylglicerol, hs-CRP, reactive hiperemia index, leptin, insulin, HOMA-IR, adiponectin and PTH) were log transformed to improve normality. Stata 10.0 (STATA Corp., College Station, TX, USA) was used for statistical analysis and $p < 0.05$ was considered statistically significant.

Ethics

The study protocol was approved by the committee on ethics and research of the Pedro Ernesto University Hospital (1152-CEP/HUPE – CAAE: 0039.0.228.000-08) and all procedures were performed in accordance with the Helsinki Declaration of 1975, which was revised in 1983. All participants provided written informed consent.

Results

Seventy three women completed all evaluations and were included in the statistical analysis.The participants presented a mean age of 32.14 ± 1.10 years, their 25(OH)D serum levels were 25.52 ± 1.32 ng/ml and average BMI was 25.86 ± 0.67 Kg/m^2. Both groups (with and without vitamin D deficiency) were comparable in several demographic characteristics (Table I), in variables related to calcium and vitamin D metabolism (Table I) and in nutrient composition of food intake (Table II).The only difference between the two groups was serum levels of 25(OH)D (Table I).

Participants with vitamin D deficiency compared with those without vitamin D deficiency exhibited significantly higher values of BMI and percentage of body fat (Table III). However after adjustment for age the difference between groups was no longer significant (Table III). WC, hip circumference, waist-to-rip ratio and waist-to-height ratio were higher in the group with vitamin D deficiency than in the group without vitamin D deficiency, although without reaching statistical significance (Table III).

Comparative analysis of the biochemical variables between the two groups showed significantly higher levels of glucose and HOMA-IR in the vitamin D deficiency group even after adjusting confounding factors (age; BMI; WC; intake of energy, protein, carbohydrates, lipids and calcium; intracellular calcium; and serum levels of PTH, calcitriol and calcium) (Table IV). The serum levels of leptin were greater in subjects with vitamin D deficiency than in subjects without deficiency, although this difference was no more significant after adjustment for age (Table IV).Both groups presented similar values of insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, hs-CRP, adiponectin and blood pressure (Table IV).

The endothelial function evaluated by reactive hiperemia index was significantly worse in participants with vitamin D deficiency than in the others: 1.68 ± 0.10 vs. 2.17 ± 0.10; p=0.01. Even after controlling for some confounding factors (age, BMI, WC, glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hs-CRP) the difference remained significant (p=0.03). However after additional adjustments for dietary calcium, intracellular calcium and serum levels of PTH, calcitriol and calcium the difference was no more significant (p=0.06) (Figure 1).

Discussion

In the present study, based on a sample of healthy Brazilian premenopausal women stratified in two

Table I
Characteristics and variables related to calcium and vitamin D metabolism of the participants according to vitamin D status

	Vitamin D status		
	Without deficiency (n = 61)	*With deficiency (n = 12)*	*P*
Age (years)	30.36 ± 1.09	36.49 ± 3.24	0.08
Non white (n; %)	18 (30%)	5 (42%)	0.41
Alcohol intake (n; %)	7 (11%)	3 (25%)	0.21
Dietary calcium intake (mg/day)	671.04 ± 36.31	686.42 ± 161.33	0.15
Serum creatinine (mg/dl)	0.84 ± 0.03	0.83 ± 0.05	0.88
Total serum proteins (g/dl)	6.99 ± 0.09	6.99 ±0.12	0.98
Serum albumin (g/dl)	3.91 ± 0.05	4.01 ± 1.00	0.40
Serum globulin (g/dl)	3.08 ± 0.06	3.15 ± 0.17	0.63
Intracellular calcium (mEq/L/Cell)	9.41 ± 0.87	8.91 ± 1.34	0.81
Total serum calcium (mg/dl)	9.23 ± 0.10	9.43 ± 0.15	0.38
Ionized serum calcium (mg/dl)	4.08 ± 0.04	4.19 ± 0.08	0.24
24h urine calcium/creatinine (mg/mg)	0.17 ± 0.01	0.16 ± 0.04	0.85
Serum parathormone (pg/ml)	5.75 ± 0.33	5.51 ± 0.72	0.60
1,25 $(OH)_2$ D (pg/ml)	197.93 ± 14.34	201.70 ± 33.82	0.92
25(OH) D (ng/ml)	31.76 ± 1.61	16.03 ± 0.67	<0.01

Values are expressed as mean ± standard error or number of participants (percentage).

Table II
Nutrient composition of food intake evaluated by the food frequency questionnaire according to vitamin D status

	Vitamin D status		
	Without deficiency (n = 61)	*With deficiency (n = 12)*	*P*
Energy (Kcal/day)	1539.32 ± 83.78	1534.54 ± 272.78	0.36
Protein (g/day)	78.56 ± 4.99	81.34 ± 8.71	0.46
Carbohydrate (g/day)	185.40 ± 9.81	184.97 ± 49.95	0.07
Lipids (g/day)	54.21 ± 3.55	53.11 ± 6.26	0.97
Dietary calcium (mg/day)	671.04 ± 36.31	686.42 ± 161.33	0.15

Values are expressed as mean ± standard error

groups on the basis of their serum levels of 25(OH) D (with vitamin D deficiency and without vitamin D deficiency), the main findings were that subjects with vitamin D deficiency presented significantly higher glucose levels and insulin resistance independent of confounding factors.

The mean value of serum 25(OH)D observed in this study was 25.52 ± 1.32 ng/ml, which is lower than the levels considered as vitamin D sufficiency (≥ 30 ng/ml)[1,6,28,29]. In a cross-sectional study conducted in São Paulo, southeastern Brazil, the mean serum concentration of 25(OH)D was even lower than in the present study: 19.36ng/ml (48.4 nmol/L) in adult men and 20.40ng/ml (51.0 nmol/L) in adult women[29].

Although Brazil is a sunny country, these low serum concentrations of 25(OH)D may have some possible explanations: (a) the sun exposure habits of the Brazilian population are not enough to maintain vitamin D adequacy and (b) in Brazil, there is no mandatory food fortification with vitamin D[29].

In the present study, participants with vitamin D deficiency compared with those without deficiency of this vitamin presented significantly higher levels of BMI and percentage body fat only before adjustment

Table III
Parameters of nutritional state according to vitamin D status

	Vitamin D status					
	Without deficiency (n = 61)	*With deficiency (n = 12)*	*P*	*P**	*P***	*P****
Body weight (kg)	64.48 ± 1.75	72.55 ± 4.04	0.07	0.32	0.27	0.17
Body mass index (kg/m^2)	25.12 ± 0.68	28.67 ± 1.45	0.04	0.24	0.20	0.15
Body fat (%)	31.09 ± 0.72	35.31 ± 1.75	0.02	0.19	0.18	0.22
Waist circumference (cm)	83.54 ± 1.75	91.39 ± 4.11	0.09	0.45	0.35	0.25
Hip circumference (cm)	100.72 ± 1.18	104.94 ± 2.69	0.10	0.56	0.57	0.48
Waist-to-hip ratio	0.83 ± 0.01	0.87 ± 0.02	0.09	0.51	0.32	0.24
Waist-to-height ratio	0.52 ± 0.01	0.58 ± 0.03	0.05	0.40	0.32	0.26

Values are expressed as mean ± standard error
* = after adjustment for age
** = after adjustment for age and intake of energy, protein, carbohydrates and lipids
*** = after adjustment for age; body mass index; waist circumference; intake of energy, protein, carbohydrates, lipids and calcium; intracellular calcium; and serum levels of parathormone, calcitriol and calcium.

Table IV
Biochemical variables and blood pressure levels according to vitamin D status

	Vitamin D status					
	Without deficiency (n = 61)	*With deficiency (n = 12)*	*P*	*P**	*P***	*P****
Glucose (mg/dl)	80.15 ± 1.13	88.25 ± 3.24	0.02	0.02	0.06	0.03
Insulin (μU/ml)	22.25 ± 1.13	26.68 ±3.69	0.14	0.23	0.38	0.55
HOMA-IR	4.42 ± 0.25	6.43 ± 0.73	0.008	0.01	0.02	0.04
Total cholesterol (mg/dl)	189.33 ± 5.10	194.33 ± 12.34	0.69	0.67	0.68	0.96
HDL-cholesterol (mg/dl)	57.70 ± 1.66	55.17 ± 2.24	0.51	0.69	0.96	0.94
LDL-cholesterol (mg/dl)	111.37 ± 4.36	117.42 ± 11.18	0.58	0.68	0.56	0.99
Triglycerides (mg/dl)	96.87 ± 5.67	108.25 ± 11.57	0.29	0.65	0.64	0.69
Leptin (ng/ml)	20.86 ± 1.74	29.56 ± 3.53	0.02	0.16	0.48	0.69
Adiponectin (μg/ml)	34.18 ± 2.56	32.33 ±4.03	0.94	0.94	0.77	0.58
hs-CRP (mg/dl)	0.45 ± 0.09	0.40 ± 0.09	0.71	0.79	0.63	0.62
Systolic BP (mmHg)	102.86 ± 1.39	110.17 ± 5.07	0.16	0.36	0.68	0.70
Diastolic BP (mmHg)	68.61 ± 1.27	72.83 ± 3.99	0.21	0.71	0.95	0.91
Mean BP (mmHg)	79.72 ± 1.27	84.30 ± 4.53	0.20	0.54	0.84	0.91
Heart rate (bpm)	74.75 ± 1.20	77.56 ± 2.59	0.33	0.28	0.16	0.06

Values are expressed as mean ± standard error
* = after adjustment for age
** = after adjustment for age and intake of energy, protein, carbohydrates and lipids
*** = after adjustment for age; body mass index; waist circumference; intake of energy, protein, carbohydrates, lipids and calcium; intracellular calcium; and serum levels of parathormone, calcitriol and calcium.

for confounders such as age.As obesity is a risk factor for vitamin D deficiency[28], probably because vitamin D is sequestered in the adipose tissue[12], it would be expected that the difference between groups remained significant even after controlling for confounding factors. One possible explanation is that in our total group of participants (n=73) only 19 women were obese and mean BMI was relatively low (25.70 ± 5.39 kg/m^2).

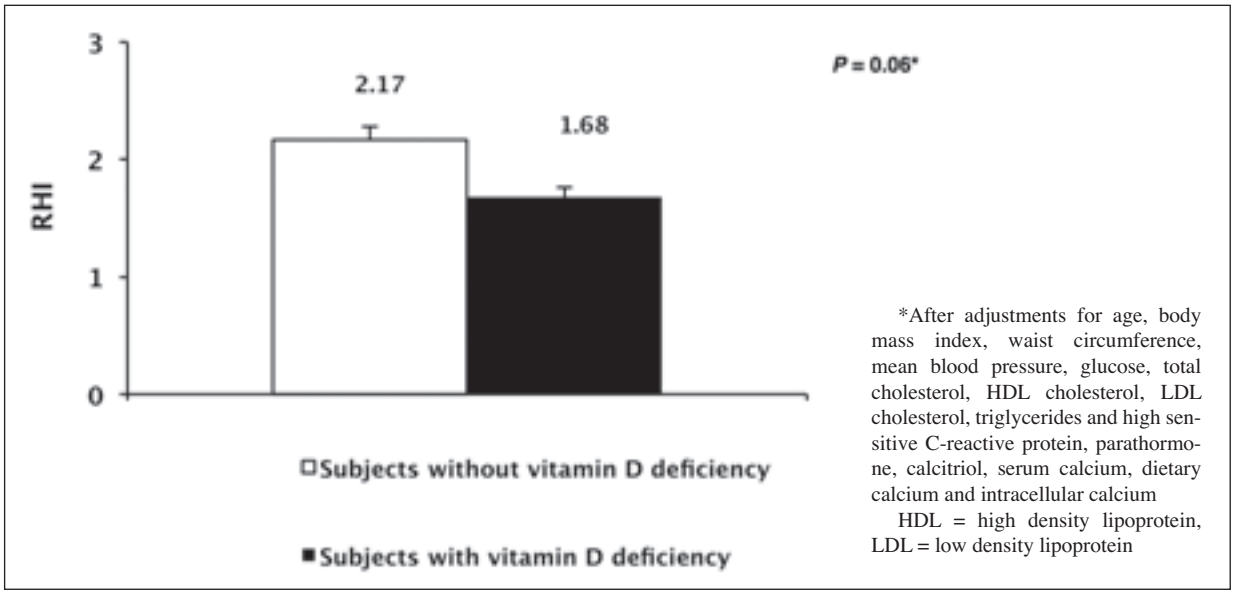

Fig. 1.—Mean levels of reactive hyperemia index (RHI) according to vitamin D status.

Insulin resistance (HOMA-IR) was greater in the group with vitamin D deficiency than in the other group, in the present study. Even after adjustments for important confounders, such as BMI and WC, this difference remained significant.As all participants included in this study were sedentary, it was not necessary to adjust our results to physical activity.Some cross-sectional studies also observed that serum 25(OH)D was inversely associated with fasting glucose and glycated haemoglobin[30,31]. Low serum levels of 25(OH)D were associated with an increased risk of type 2 diabetes in prospective studies[6,32,33]. In a recent meta-analysis, involving 76,220 participants, the highest compared with the lowest category of 25(OH)D levels presented a relative risk for type 2 diabetes of 0.62 (95%CI 0.54 – 0.70).A linear trend analysis showed that each 10mmol/L (4ng/ml) increase in 25(OH)D levels was associated with a decrease of 4% in the risk of type 2 diabetes[34].

Several mechanisms that might link vitamin D to impaired glucose metabolism have been proposed, including abnormalities in insulin secretion and action. Vitamin D may stimulate insulin release by pancreatic β cells and may also act on insulin action by stimulating the expression of insulin receptors and amplifying glucose transport. These effects of vitamin D may be directly mediated by the biding of $1,25(OH)_2D$ to its receptor or may be indirect through elevated PTH levels or alterations in intracellular cytosolic calcium, both consequent to low serum levels of 25(OH)D. Nowadays, vitamin D is considered to have an anti-inflammatory effect and this could ameliorate low-grade chronic inflammation that has been implicated in insulin resistance in type 2 diabetes[35,36]. The present study suggests that 25(OH)D may interfere in insulin resistance independently of PTH and intracellular calcium. Fraser et al (2010) also observed that the associations of 25(OH)D with fasting glucose and insulin were independent of serum PTH and calcium[37].

In the present study the endothelial function of women with vitamin D deficiency was worse than that of the participants without deficiency only before controlling for variables involved in calcium metabolism. Endothelial dysfunction is considered one of the possible mechanisms for the inverse association between serum 25(OH)D and the risk of CV events and mortality found in observational studies[1,2].

The findings of the present study suggest that, in healthy premenopausal women, vitamin D deficiency is associated with insulin resistance independent of dietary calcium, intracellular calcium and serum levels of parathormone, calcitriol and calcium.

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