Vitamin D and Endothelial Vasodilation in Older Individuals: Data From the PIVUS Study

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Context: Vitamin D plays a role in a wide range of extraskeletal processes, including vascular function. Endothelial dysfunction is a predictor of cardiovascular disease, especially in older subjects. However, the relationship between vitamin D levels and indexes of endothelial vasodilation has never been fully addressed in older individuals.

Objective: The objective of this study was to examine the association between vitamin D and endothelial function in a large community-based sample of older subjects.

Methods: This cross-sectional study involved 852 community-dwelling men and women aged 70 years from the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS), with complete data on vascular function and 25-hydroxyvitamin D. We evaluated endothelium-dependent vasodilation by an invasive forearm technique with acetylcholine, endothelium-independent vasodilation by sodium nitroprussiate, flow-mediated vasodilation, and the pulse wave analysis (reflectance index). Vitamin D levels were measured by chemiluminescence. We used multivariate regression models adjusted for body mass index (model 1) and for multiple confounders (high-sensitivity C-reactive protein, insulin, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, smoking, sex hormones, season of blood collection, hypertension, diabetes, cardiovascular medications and diseases, statin usage, plasma calcium and calcium intake, PTH, physical exercise, liver and kidney function tests, albumin; model 2).

Results: In women, but not in men, vitamin D levels were positively associated with endothelium-independent vasodilation in both model 1 (β ± SE = 1.41 ± 0.54; P = .001), and model 2 (β ± SE = 2.01 ± 0.68; P = .003). We found no significant relationship between vitamin D levels and endothelium-dependent vasodilation, flow-mediated vasodilation, and reflectance index in both sexes.

Conclusions: In older women, but not in men, vitamin D is positively and independently associated with EIDV. (J Clin Endocrinol Metab 99: 3382–3389, 2014)
In the aging population, a physiological gradual decline of vitamin D levels is frequently observed (1). Vitamin D deficiency, historically defined and recently recommended by the Institute of Medicine as 25-hydroxyvitamin D (25-OH D) serum levels of less than 50 nmol/L, is a common condition in older persons, affecting 20–100% of US, Canadian, and European elderly men and women still living in the community (2, 3).

Specific lifestyle factors linked to advanced age concur to determine a poor vitamin D status. These include poor dietary intake of vitamin D, chronic diseases, insufficient exposure to sunlight, pharmacological treatment, obesity, and disability (1).

A suboptimal vitamin D status represents an important potential public health issue because it has been associated with an increased risk of chronic conditions, cardiovascular events, disorders of glucose metabolism, neurodegenerative diseases, and overall mortality (4).

The consequences of vitamin D deficiency can be explained by the fact that this hormone not only regulates calcium and phosphate balance and bone structure, but also plays an important role in a wide range of extraskelatal biological functions via both genomic and non-genomic signaling (5). In particular, recent evidence suggests that vitamin D could influence vascular biology by modulating endothelial function and inflammatory status (6). It is well established that endothelial dysfunction is a hallmark of vascular damage and an independent predictor of atherosclerosis and cardiovascular events (7).

Observational studies reported that healthy middle-aged 25-OH D-deficient subjects are more prone to develop arterial stiffness and endothelial dysfunction (6, 8). A number of biological hypotheses have been formulated to explain the beneficial effects of vitamin D at the vascular level (6). However, the exact mechanisms need to be clarified. Experimental evidence suggests that vitamin D could affect the vascular wall through its binding to the vitamin D receptor (VDR), which is expressed on at least 36 different tissues including cardiac muscle, vascular smooth muscle, vascular endothelium, and lymphocytes (9, 10). In vitro studies in human umbilical vein endothelial cells have shown that vitamin D metabolites are capable of influencing endothelium-dependent vasodilation (EDV) by increasing nitric oxide synthase activity and nitric oxide production (11). Vitamin D, in particular, seems to interact with the renin-angiotensin-aldosterone axis (12, 13) as well as with vascular endothelial growth factor production. Finally, vitamin D might also exert antithrombotic effects by reducing platelet aggregation (14) and altering the production of proteins involved in the regulation of thrombogenesis, eg, thrombomodulin and antithrombin (15).

Despite the potential link between vitamin D and endothelial function, the relationship between vitamin D levels and endothelial vasodilation has never been fully addressed in older individuals.

This is a significant issue because older adults are more susceptible to both vitamin D insufficiency and increased risk of cardiovascular diseases (16). Thus, our study was designed to examine the relationship between serum 25-OH D and indexes of vascular function using data from a unique community-based sample of older men and women with complete information on serum 25-OH D levels, endothelium-independent vasodilation (EIDV), and EDV assessed by three different methods: an invasive forearm technique with acetylcholine, flow-mediated vasodilation (FMD), and pulse wave analysis.

Subjects and Methods

Population
To address our original hypothesis, we studied participants aged 70 years from the large population-based Prospective Study of the Vasculature in Uppsala Seniors (PIVUS).

All subjects aged 70 years and living in the community of Uppsala, Sweden, were eligible. The study population, chosen from the registry of community-living individuals, was invited in a randomized order. The subjects received an invitation by letter within 2 months of their 70th birthday. Of the 2025 subjects invited, 1016 subjects participated (507 men and 509 women), yielding a participation rate of 50.1%. The baseline investigation started in April 2001 and was completed in June 2004. From the entire sample of 1016 subjects, we analyzed data of 852 subjects, 428 men and 424 women, who had complete information on vitamin D and measures of endothelial function assessed with three different methods.

The study protocol was approved by the Ethics Committee of the University of Uppsala. All participants received a detailed description of the purpose and design of the study and signed an informed participation consent.

Baseline clinical investigations
The participants were asked to answer a complete questionnaire about their medical history, regular medication, and dietary and smoking habits. Specifically, subjects gave information about the history of any cardiovascular diagnosis or medication, hypertension (antihypertensive treatment or blood pressure > 140/90 mm Hg), diabetes (antidiabetic treatment including diet or fasting blood glucose > 6.1 mmol/L), hyperlipidemia (antihyperlipidemic treatment, low-density lipoprotein [LDL]-cholesterol > 3.5 mmol/L, or serum triglycerides > 1.7 mmol/L). Alcohol, vitamin D, and calcium intake were assessed with a 7-day food diary. Participants were instructed in person by trained dieticians on how to fill in the 7-day precoded food diary. Daily nutrient intake was calculated using a computer program and the Swedish National Food Administration database (SLV Database, 1990). Blood pressure was measured with a calibrated mercury sphygmomanometer at least at 30 minutes of rest, and the average of three recordings was used. Standing height
was measured to the nearest whole 0.5 cm with a Harpenden Stadiometer (Holtain Ltd), and body weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated as body weight/(height)^2 (kg/m^2), and obesity was defined as BMI > 30 kg/m^2. Waist circumference was measured in a standing position midway between the lowest rib and the iliac crest. Exercise habits were divided into four categories: very light exercise (no sweat) less than two times per week, light exercise two times per week, moderate exercise (sweat) one or two times per week, and heavy exercise (sweat) more than two times per week. Because of the known seasonal variability in vitamin D concentration, the season for blood collection was considered as a categorical season variable with 3-month intervals. Season categories were identified as follows: winter (December to February), spring (March to May), summer (June to August), and fall (September to November).

**Laboratory investigations**

Blood was collected in the morning, after an overnight fast. All routine blood tests were performed on fresh blood. No medication or smoking was allowed after midnight. Plasma calcium (normal range, 2.20–2.50 mmol/L) was measured spectrophotometrically with a complexometric method using ortho-cresolphthalein dye binding. Liver function was assessed by plasma alanine aminotransferase (ALT; normal range, 0–40 U/L) and plasma albumin (spectrophotometry using bromine cresol purple; normal range, 37–48 g/L). Plasma creatinine was analyzed with spectrophotometry using a modified Jaffe's reaction (normal range, 60–106 μmol/L). Creatinine clearance (normal range in men, 97–137 mL/min; in women, 88–128 mL/min) was calculated using the Cockcroft-Gault formula: for men ≥20 years of age, creatinine clearance = [1.23 × (140 – age) × weight]/S-creatinine; for women ≥20 years of age, creatinine clearance = [1.04 × (140 – age) × weight]/S-creatinine. Total plasma cholesterol (normal range, 2.6–7.1 mmol/L) and plasma high-density lipoprotein (HDL)-cholesterol (normal range, 0.8–1.9 mmol/L) were determined by enzymatic assay. LDL-cholesterol was calculated using Friedewald's formula. High-sensitivity C-reactive protein (hsCRP) was measured in serum by latex-enhanced turbidimetry on an Architect Ci8200 analyzer (Abbott Laboratories).

**Hormonal assessment**

All of the study samples were measured in one laboratory that takes part in, and meets the performance targets for the Vitamin D External Quality Assessment Scheme (DEQAS). 25-OHD was measured in serum by chemiluminescence immunoassay technology (LIAISON 25-hydroxyvitamin D Assay; DiaSorin) (17). The coefficient of variation for interassay analyses was 18.4% at a 25-OHD level of 39.5 nmol/L and 11.7% at 121.25 nmol/L. Severe vitamin D deficiency was defined as serum 25-OH D levels < 25 nmol/L. Vitamin D deficiency was defined as serum 25-OH D levels < 50 nmol/L. Vitamin D sufficiency was defined as serum 25-OH D levels > 75 nmol/L. Intact plasma PTH (normal range, 12–65 ng/L) was determined with an immunochemiluminometric assay (Nichols Institute). Serum SHBG, estradiol (E2), and T were assessed by chemiluminescence immunoassay on the immunochemistry platform Access (Beckman Coulter). The minimal detectable concentrations for E2, T, and SHBG were 73 pmol/L, 0.35 nmol/L, and 2 nmol/L, respectively. The coefficient of variation was < 20% for E2, < 7% for T, and < 6% for SHBG, respectively. Plasma insulin was assayed by using chemiluminescence (Roche).

**Measures of endothelial function**

Three different techniques to evaluate EDV in the peripheral circulation were simultaneously used in this large-scale population-based setting. All three techniques were feasible to perform in a general elderly population. Detailed information has been previously described elsewhere (18).

Briefly, the forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedica). After evaluation of resting FBF, local intra-arterial drug infusions were given over 5 minutes for each dose. The infused dosages were 25 and 50 μg/min for acetylcholine (Clin-Alpha) to evaluate EDV, and 5 and 10 μg/min for sodium nitroprussiate (Nitropress; Abbott) to evaluate EIDV. EDV mainly reflects vasodilation in forearm resistance arteries. FBF was defined as FBF during infusion of 50 μg/min of acetylcholine minus resting FBF divided by resting FBF. EIDV was defined as FBF during infusion of 10 μg/min of sodium nitroprussiate minus resting FBF divided by resting FBF. FMD was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 30 mm Hg above the subject's systolic blood pressure for 5 minutes, and defined as the maximal brachial artery diameter recorded between 30 and 90 seconds after cuff release minus diameter at rest divided by the diameter at rest. The brachial artery diameter was measured by external B-mode ultrasound imaging 2–3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer; Acuson), according to the recommendations of the International Brachial Artery Task Force (19). The pulse wave analysis was assessed with a micromanometer-tipped probe (SphygmoCor; Pulse Wave Medical Ltd) applied to the surface of the skin overlying the radial artery, and the peripheral radial pulse wave was continuously recorded. The mean values of 10 pulse waves were used for analyses. After a baseline recording, terbutaline was administered sc (0.25 mg in the upper part of the arm) and a reevaluation of the pulse wave was performed after 15 and 20 minutes. The maximal change occurring at either 15 or 20 minutes was used for calculations. This technique mainly evaluates vasodilation in resistance arteries. Reflectance index (RI) is the measure assessed by the pulse wave-based technique.

**Assessment of body composition**

Total body fat content was assessed by dual-energy x-ray absorptiometry (DXA) (DPX, Lunar Prodigy; Lunar Corp), with the primary outcome of total body fat mass. To evaluate the reproducibility, 15 subjects were scanned three times. The coefficient of variation of the DXA measurements was calculated to be 1.5% for total fat mass and 1.0% for total lean mass (20). The bias associated with DXA fat measurement is systematic, with an underestimation of fat content for leaner subjects and an overestimation of fat content among obese subjects, but these inaccuracies were less than 2% (21).

**Statistical analysis**

Categorical variables were expressed in percentages, and continuous variables were reported as means (SD) for normally distributed parameters or as medians and interquartile ranges. To approximate normal distributions, log-transformed values for FMD, RI, EDV, EIDV, ALT, creatinine clearance, hsCRP, insu-
lin, T, E2, SHBG, PTH, calcium intake, and total body fat were used in the analysis and back-transformed for data presentation.

The main effects of vitamin D on endothelial function in both sexes were tested in a BMI-adjusted analysis (model 1). The interaction term sex×vitamin D was used in a general linear model to compare the sex-associated increments in endothelial function and vitamin D between sexes. Because the interaction term sex×vitamin D was significant in the relationship between vitamin D and EIDV, the models were performed separately for men and women. Parsimonious models obtained by backward selection from initial fully adjusted models were used to identify independent correlates of endothelial function in both sexes (model 2). Model 2 was adjusted for BMI and other potential confounders including hsCRP, creatinine clearance, ALT, albumin, plasma calcium, calcium intake, PTH, insulin, T, E2, SHBG, total cholesterol, HDL-cholesterol, LDL-cholesterol, smoking, season of blood collection, diabetes, hypertension, cardiovascular diseases (angina, stroke, myocardial infarction, coronary artery bypass surgery), any cardiovascular drugs, statin use, and physical exercise. A statistical significance was set for \( P < .05 \). The analyses were performed using the SAS statistical package, version 9.1 (SAS Institute Inc).

Results

Characteristics of the population

The characteristics of the study population are presented in Table 1. Data are presented as mean SD, or median [interquartile range]. As expected, when compared to men, women had significantly lower waist circumference (86.8 ± 10.8 vs 93.9 ± 9.9 cm; \( P < .001 \)), E2 (64 [49–87] vs 109 [90–132] pg/mL; \( P < .001 \)), T (0.41 [0.14–0.80] vs 12.8 [10.1–15.6] mmol/L; \( P < .001 \)), and vitamin D intake (5.30 ± 1.85 vs 6.24 ± 2.35 \( \mu \)g/d; \( P < .001 \)). Women had higher levels of total cholesterol (5.7 ± 0.96 vs 5.1 ± 0.91 mmol/L; \( P < .001 \)), HDL-cholesterol (1.7 ± 0.4 vs 1.4 ± 0.4 mmol/L; \( P < .001 \)), LDL-cholesterol (3.54 ± 0.84 vs 3.27 ± 0.83 mmol/L; \( P < .001 \)), SHBG (55.5 [42.1–73.8] vs 42.1 [32.4–54.2] nmol/L; \( P < .001 \)), and total body fat (26.83 [20.99–33.53] vs 22.53 [17.71–27.91] g/cm\(^2\); \( P = .001 \)). RI was significantly different in women (–34.8 [–44.3 to –25.6]) and men (–27.9 [–37.1 to –10.9]), and this difference was statistically significant (\( P < .001 \)). Alcohol intake (g/d), was only available in 367 of 428 men and 354 of 424 women. Men had a significantly higher alcohol intake than women (2.34 [0.65–4.67] vs 1.16 [0.22–2.8] g/d; \( P < .001 \)) (data not shown). Men and women had similar mean serum 25-OH D levels (\( P = .55 \)).

Interaction between sex, vitamin D, and endothelial function

By analyzing the interaction between sex and vitamin D, we found a statistically significant interaction term regarding EIDV (vitamin D×sex, \( \beta \pm SE, 1.50 \pm 0.75; P = .04 \)). No significant interaction between vitamin D×sex was found for EDV (\( \beta \pm SE, 0.70 \pm 1.0; P = .48 \)), FMD (\( \beta \pm SE, 0.12 \pm 0.33; P = .70 \)), and RI (\( \beta \pm SE, –0.02 \pm 0.05; P = .57 \)).

Vitamin D and endothelial function measures in women

After adjustment for BMI, we found a positive relationship between vitamin D and EIDV in women (\( \beta \pm SE = 1.41 \pm 0.54; P = .001 \)) (Table 2). The relationship between vitamin D and EIDV was maintained after further adjustment for hsCRP, creatinine clearance, ALT, albumin, plasma calcium, calcium intake, insulin, T, E2, SHBG, total cholesterol, HDL-cholesterol, LDL-cholesterol, hypertension, smoking, season of blood collection, diabetes, any cardiovascular drugs, statin use, calcium intake, PTH, physical exercise, and cardiovascular diseases (\( \beta \pm SE = 2.01 \pm 0.68; P = .003 \)) (Table 2).

The association was still significant after further adjustment for total body fat (\( \beta \pm SE = 1.98 \pm 0.66; P = .003 \)) and vitamin D intake (\( \beta \pm SE = 1.47 \pm 0.72; P = .04 \)) (data not shown). Similarly, in a restricted sample of 721 subjects having complete data on alcohol intake, the inclusion of this variable in the multivariate regression model did not affect the positive and significant relationship between vitamin D levels and EIDV (\( \beta \pm SE = 1.55 \pm 0.75; P = .004 \)) (data not shown).

No significant association was observed between vitamin D and EDV, FMD, and RI in model 1 and model 2 (Table 2).

Vitamin D and endothelial function measures in men

In men, we did not detect any significant relationship between vitamin D and EIDV using model 1 (\( \beta \pm SE = 0.02 \pm 0.49; P = .95 \)) and model 2 (\( \beta \pm SE = –0.28 \pm 0.61; P = .65 \)) (Table 3). Similarly, no significant association was found between vitamin D and all measures of endothelial function in the BMI and fully adjusted model (Table 3). These results were not affected by further adjustment for total body fat (\( \beta \pm SE = –0.18 \pm 0.58; P = .75 \)) and vitamin D intake (0.03 ± 0.62; \( P = .95 \)) (data not shown).

Discussion

In a large community-based population of older subjects, we found an independent positive association between vitamin D levels and EIDV in women but not in men.

Our findings demonstrate a profound link between vitamin D and measures of EIDV by also providing novel
insights into the extraskeletal effects of vitamin D on the vascular system. To our knowledge, this is the first study that has addressed the relationship between vitamin D and endothelial function in a population of older individuals with complete information on the most relevant indexes of endothelial vasodilation and arterial stiffness.

Most of the previous investigations, conducted in small cohorts of middle-aged subjects, focused on the impact of vitamin D on surrogate markers of vascular disease (particularly blood pressure) and noninvasive measures of EDV (7, 22).

The existing cross-sectional data coming from healthy individuals and patients with different diseases suggest an association between vitamin D deficiency and endothelial dysfunction, arterial stiffness, and a broad range of cardiovascular disorders and risk factors (2, 3). Wang et al (23) showed an increased risk of cardiovascular events in participants without overt cardiovascular disease having 25-OH D concentrations < 37.5 nmol/L. Interestingly, the risk doubled in those participants with the lowest 25-OH D concentrations. In a recent analysis of a well-represented study population of 514 subjects aged

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Data are presented as number of cases (percentage), mean ± SD, or median [interquartile range] as appropriate.

a Log-transformed values.

b Seasonal categories were identified as follows: winter (from December to February), spring (from March to May), summer (from June to August), and fall (from September to November).

c Very light exercise, no sweat <2 times per week.

d Light exercise, no sweat >2 times per week.

e Moderate exercise, with sweat, <2 times per week.

f Heavy exercise, with sweat, >2 times per week.
Cardiovascular drug, statin usage, calcium intake, PTH, physical season of blood collection, E2, T, SHBG, hypertension, diabetes, any insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, smoking, exercise, angina, stroke, and myocardial infarction.

47 ± 13 years, Al Mheid et al (24) demonstrated that lower 25-OH D levels were associated with worse endothelium-dependent brachial artery FMD and arterial stiffness assessed by pulse wave velocity. These results were maintained after adjustment for age, sex, race, BMI, serum lipid levels, plasma hsCRP, and medications. Impaired FMD was also reported in chronic diseases such as type 2 diabetes (25). Vitamin D seems to act locally in vascular smooth muscle and endothelial cells through its binding with the VDR. In these tissues vitamin D might modulate the effects of inflammatory cytokines on the vasculature (26), decrease endothelial adhesion molecules expression, increase nitric oxide production (12), and reduce platelet aggregation (16, 27).

However, in the present analysis we failed to detect any significant association between vitamin D and EDV, FMD, and RI. To explain the discrepancy between our data and the previous investigations, we suggest that factors other than vitamin D might be more relevant in modulating endothelial function in older individuals (28). Moreover, as observed for most of the other nuclear receptors, there is a reduction in the number and/or expression of VDRs associated with aging (29). In older subjects, low serum levels of vitamin D may significantly reduce VDR activation and function (30). These observations provide evidence for the notion of an age-related relative insensitivity of EDV to vitamin D.

EIDV, evaluated by invasive forearm technique, is considered a reliable marker of the typical age-related changes in vascular structure (loss of elastin fibers, increased collagen fibers and smooth muscle cells in the media layer) and vascular stiffness (18). This index has been significantly related to the Framingham risk score and proinflammatory and prothrombotic states (18, 31). Several lines of evidence suggest that vitamin D may indirectly modulate endothelium-independent function by suppressing the renin-angiotensin system, reducing blood pressure (32), and/or decreasing vascular resistance (33).

More interestingly, the findings of the present analysis also suggest that low vitamin D is, at least in women, a marker of poor health and overall metabolic status, rather than the cause of vascular and physiological disturbances. Low 25-OH D could be the result of vascular diseases and a useful marker in the detection of functional and vascular damage before its clinical manifestation. This hypothesis was recently raised in a systematic review documenting a discrepancy between the existing observational and interventional studies on the role of 25-OH D concentrations in a wide range of acute and chronic nonskeletal health disorders (16). Randomized trials have failed to demonstrate that raising of 25-OH D concentrations can modify the occurrence or clinical course of vascular disorders (22). Well-designed trials performed in postmenopausal women using low (400 IU) (34) and high (2500 IU) daily vitamin D doses (35) did not show any improvement either in EDV or in measures of arterial stiffness. Overall, these data do not support a causal relationship between low vitamin D status and a wide range of disorders (16). Interestingly, the relationship between vitamin D and EIDV was independent of hsCRP and severe cardiovascular and cerebrovascular diseases, supporting the hypothesis that vitamin D may be more a biomarker of metabolic status rather than inflammatory diseases.

We also found a specific sex-related influence of vitamin D on EIDV. These results are confirmed by the statistically significant interaction found between sex and vitamin D for EIDV. To identify potential mediators of this sex-specific association, we determined the role of sex hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. All of these variables are known to have different hormones such as T and E2 and the transport protein SHBG. 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explaining the different results in men and women can be found in the physiological and age-related differences in obesity and body fat distribution existing between the two sexes (36, 37). As expected, in our population men had higher waist circumference than women. We also know that vitamin D is a fat-soluble vitamin and lower levels are encountered in obese and diabetic patients (38, 39). To exclude the influence of body fat content, we have also included total body fat as confounder; but, again, the association between vitamin D and EIDV in women was still statistically significant.

Limitations and strengths

The cross-sectional design of the study does not allow us to establish a cause-and-effect relationship between vitamin D levels and EIDV in the older female cohort. The sample included in our analysis was comprised of white persons aged 70 years old, most of whom were taking cardiovascular drugs. We acknowledge that the study results might be affected by the relatively small number of study subjects. However, it is not easy to perform invasive methods of endothelial function assessment, especially in such populations of older individuals. To minimize bias, we accounted for metabolic and cardiovascular confounding variables. However, given the significant number of differences between female and male participants, particularly in terms of total, HDL-, and LDL-cholesterol levels, total body fat, BMI, and liver and kidney function, we cannot exclude the compounding effects of these variables in the relationship between vitamin D and EIDV. Moreover, although the DiaSorin Liaison immunoassay, used in the current analysis, is the most common technique in the DEQAS validation program, we acknowledge that there are other methodologies such as liquid chromatography-tandem mass spectrometry, which is considered the “gold standard” in the assessment of 25-OH D levels. Finally, based on the above-mentioned limitations and given the difficulty of comparing our findings with the information currently available in the literature, we cannot exclude the possibility of the chance finding. Thus, our study analysis can be considered a generating hypothesis that needs to be confirmed in future investigations.

These limitations are offset by the significant strengths. Our study is the first investigating the relationship between vitamin D and endothelial function, assessed by three invasive methods of evaluation, in the only existing large-scale, population-based cohort of older individuals with complete information on vitamin D levels and intake and other important variables. Finally, all the study samples were measured in one laboratory that met the performance targets for the vitamin D DEQAS (40).

Conclusion

In older women, but not in men, vitamin D concentration was positively and independently associated with EIDV. Further longitudinal analyses are needed to delineate the role of vitamin D in sex-related endothelial function and derived diseases as well as the usefulness as marker of early functional vascular damage.

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