Association of Parathyroid Hormone and 25-OH-Vitamin D Levels with Arterial Stiffness in Postmenopausal Women with Vitamin D Insufficiency

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Aim: Vitamin D insufficiency and increased parathyroid hormone (PTH) levels have been suggested as prognostic indices for cardiovascular disease. Arterial stiffness, a surrogate marker for cardiovascular disease, is often increased in patients with primary hyperparathyroidism. PTH levels increase in patients with low 25-OH-vitamin D levels, but the influence of such an increase on arterial stiffness has not been investigated in postmenopausal women with reduced 25-OH-vitamin D levels. We therefore investigated the association between PTH and aortic stiffness in postmenopausal women with reduced 25-OH-vitamin D levels.

Methods: One hundred fifty postmenopausal women with 25-OH-vitamin D insufficiency (<30 ng/mL) were recruited. Aortic pulse wave velocity (aPWV), a measure of arterial stiffness, PTH and 25-OH-vitamin D levels were measured. Cardiovascular risk factors and markers of bone formation were evaluated.

Results: The 25-OH-vitamin D levels were associated with aPWV (rho = -0.23, p = 0.006), but the association was not significant when controlling for PTH. Significant correlates of aPWV included age, body mass index, mean arterial pressure and PTH (rho = 0.39, p < 0.001). Arterial stiffness was predicted by logarithmically transformed PTH levels (β = 0.23, p = 0.007), independent of traditional cardiovascular risk factors and factors involved in bone formation. Increased PTH levels (>62 pg/mL) were associated with a 3.0-5.4-fold increased probability of having a mild-severe increase in aortic stiffness, irrespective of confounders.

Conclusion: Among postmenopausal women with reduced 25-OH-vitamin D levels, elevated PTH levels were a significant predictor of aortic stiffness, irrespective of cardiovascular risk factors and of factors involved in bone formation. PTH accounted for the association between 25-OH-vitamin D levels and aortic stiffness.


Key words: Aortic stiffness, Parathyroid hormone, Vitamin D, Postmenopausal
hyperparathyroidism\textsuperscript{9-11}. Also, Rubin \textit{et al.}\textsuperscript{12} found a positive correlation between PTH levels and aortic stiffness in patients with mild primary hyperparathyroidism; however, other studies in patients with primary hyperthyroidism did not confirm the association between PTH levels and aPWV\textsuperscript{10, 13, 14}. Moreover, the association between PTH levels and aPWV in patients with renal failure on either hemodialysis or peritoneal dialysis was null\textsuperscript{15} or even inverse\textsuperscript{16}. Notably, in patients with primary hyperparathyroidism who underwent parathyroidectomy, the decline in PTH levels after surgery was paralleled by a significant reduction in aPWV\textsuperscript{9, 11}. Also, cinacalcet, a calcimimetic that is effective in reducing serum PTH both in primary and secondary hyperparathyroidism\textsuperscript{17, 18}, ameliorated aPWV in patients with chronic renal disease and secondary hyperparathyroidism\textsuperscript{19}. Although the association between PTH levels and aPWV has been sufficiently investigated in the above-mentioned clinical settings\textsuperscript{9-19}, data are lacking on the link between PTH levels and arterial stiffness in postmenopausal women with 25-OH-vitamin D insufficiency and normal renal function.

Vitamin D insufficiency (25-OH-vitamin D < 30 ng/mL)\textsuperscript{20} is relatively common in postmenopausal women\textsuperscript{21, 22}, and recent studies have suggested an association between reduced levels of vitamin D and cardiovascular risk\textsuperscript{23, 24}. Multiple mechanisms have been proposed to explain the deleterious role of hypovitaminosis D on cardiovascular risk\textsuperscript{25}, including the negative correlation between serum vitamin D levels and arterial stiffness\textsuperscript{25, 26}. London \textit{et al.}\textsuperscript{25} found that 25-OH-vitamin D levels were negatively correlated with aPWV in patients with end-stage renal disease. In the same study, an association between PTH levels and aPWV was not detected; thus, PTH was not included as a potential confounder of the association between 25-OH-vitamin D and aPWV\textsuperscript{25}. More recently, Al Mheid \textit{et al.}\textsuperscript{26} explored the association between 25-OH-vitamin D and arterial stiffness in healthy adults. They found a negative correlation between 25-OH-vitamin D and aPWV; however, also in that study\textsuperscript{26}, the correlation between vitamin D and aPWV was not adjusted for PTH levels.

Since vitamin D insufficiency is a well-recognized cause of secondary hyperparathyroidism\textsuperscript{27-29}, and PTH levels seem to influence aortic stiffness, at least in some studies\textsuperscript{9-13}, we investigated the independent associations between 25-OH-vitamin D, PTH and aortic stiffness in postmenopausal women with vitamin D insufficiency and normal renal function.

### Study Subjects

The study population consisted of 150 postmenopausal women with vitamin D insufficiency, normal renal function (glomerular filtration rate >60 mL/min), independent in daily living activities and attending our Unit of Bone and Mineral Metabolism for screening for postmenopausal osteoporosis. Women were considered postmenopausal if they had not been menstruating for at least 1 year. Vitamin D insufficiency was defined by 25-OH-vitamin D < 30 ng/mL\textsuperscript{20}. Exclusion criteria included a history of diabetes, chronic diseases, such as thyroid, renal, hepatic, cardiac, and rheumatic diseases, current or prior use of drugs that could interfere with bone metabolism (i.e. glucocorticoids, antiresorptive drugs and hormonal replacement therapy), and a history of traumatic fractures. Forty-five patients had essential hypertension (three blood pressure measurements ≥ 140/90 mmHg or anti-hypertensive therapy) and 62 had hypercholesterolemia (LDL cholesterol ≥ 160 mg/dL or statin therapy). A trained interviewer gave each participant a questionnaire regarding age, ages related to menstrual history (menopause and menarche), smoking habits, medical history, co-morbid diseases, and medication use. Information was also obtained by reviewing the medical records and laboratory data. Calcium intake was quantified according to an Italian validated food frequency questionnaire\textsuperscript{30}. The study was approved by the local Ethics Committee and all participants gave their informed consent.

### Clinical Evaluation and Bone Mineral Density

All the determinations were made at the medical centre at 8 a.m., with room temperature between 21 and 23°C, after a 13-h overnight fast. Height and weight were measured to the nearest 0.1 cm and 0.1 Kg respectively, subjects were wearing hospital gowns and had bare feet. Body mass index was calculated as weight (kilograms) divided by height (meters) squared. Brachial blood pressure was measured by a physician with a mercury sphygmomanometer after patients had sat for 10 minutes or longer. The average of three measurements was considered for the analysis. Mean arterial pressure (MAP), as a measure of distending pressure, was calculated according to the following equation: MAP = diastolic blood pressure + (systolic blood pressure – diastolic blood pressure)/3.

Areal bone mineral density (BMD) (g/cm\(^2\); bone mineral content relative to projection area) was measured by DXA (Hologic Discovery W; Hologic Inc., Bedford, MA, USA) at the proximal femur, with a
coefficient of variation at our laboratory of 0.51%. Results for areal BMD were transformed to T scores, calculated as the difference between the actual measurement and the mean value of healthy gender-matched adult controls divided by their standard deviation.

Biochemical Assays
RIA assay was used to measure serum 25-OH-vitamin D [DiaSorin Inc., Stillwater, MN, USA]. Serum intact PTH levels were measured by an immunoenzymatic method (Access; Beckman Coulter Inc., Fullerton, CA, USA). Serum total calcium and albumin were measured by an automated chemistry analyzer. Albumin-corrected calcium was calculated as calcium + \([0.8 \times (4 – \text{serum albumin})]\), where calcium is in mg/dL and albumin is in g/dL. The bone-specific isoenzyme of alkaline phosphatase was measured by immunoradiometric assay (Tandem R Ostase; Pantec srl, Torino, Italy). Serum osteocalcin was assayed by immunometric method (DPC Immulonite, Los Angeles, CA, USA). Total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and glucose levels were determined by the enzymatic-colorimetric method (Dimension Autoanalyzer; DADE Inc., Newark, NJ, USA); LDL cholesterol was calculated by the Friedewald equation.

Measurement of aPWV
aPWV was determined using an automatic device, the SphygmoCor Vx system (AtCor, Sydney, Australia), as previously described\(^{31-33}\). It uses a single-lead ECG and a high-fidelity applanation tonometer to measure the pressure pulse waveform sequentially at two peripheral artery sites, one at the base of the neck for the common carotid artery and the other over the femoral artery. aPWV is calculated from measurements of pulse transit time and the distance between the two sites, according to the following formula: 

$$\text{PWV} \ (\text{m/s}) = \frac{\text{distance} \ (\text{m})}{\text{transit time} \ (\text{s})}$$

The numerator is the distance between the suprasternal notch and the femoral artery minus the distance between the carotid sampling site and the suprasternal notch; the denominator is the time interval between the systolic R wave and the femoral systolic up-stroke minus the time interval between the systolic R wave and the carotid systolic up-stroke. The distance between the two sites is measured using a standard compass system, thus avoiding having the measurement influenced by thoracic and abdominal profiles. An average of 10 different cardiac cycles on each site was used for the analysis. All measurements were performed by the same observer, who was blinded to the patient’s characteristics. The intra-observer variability measured in 50 healthy young volunteers was 5.1%\(^{31}\).

Statistical Analysis
The SPSS statistical package, release 10.0 [SPSS Inc., Chicago, IL, USA] was used for all statistical analyses. Values are expressed as the mean ± SD. Logarithmic (LG) transformation was performed for non-parametric variables. PTH was measured by the independent-sample t test and the Wilcoxon rank-sum test was used to compare the study variables between women with either 25-OH-vitamin D < 20 ng/mL or ≥ 20 ng/mL\(^{20, 34, 35}\); the latter cut-off was chosen according to the frequent definition of vitamin D deficiency as serum 25-OH-vitamin D < 20 ng/mL\(^{20-23}\). Correlation analyses were performed using Pearson’s and Spearman’s coefficients of correlations. In Spearman’s correlation analysis, the absolute non-LG transformed values of non-parametric variables were used. A partial correlations procedure was computed to obtain partial correlation coefficients that describe the linear relationship between 25-OH-vitamin D and aPWV while controlling for the effect of PTH levels. Stepwise regression analysis was used to estimate the prediction of aPWV and included the following independent variables: age, BMI, smoking status, MAP, heart rate, glucose, LDL cholesterol, glomerular filtration rate, femur BMD, calcium intake, 25-OH-vitamin D and LG-PTH. Also, either the hypertensive and hypercholesterolemic status, or anti-hypertensive and statin therapies were entered in multivariate analysis; further adjustment for bone specific alkaline phosphatase, osteocalcin, albumin-corrected calcium levels and month of examination [categorical; modeled as dark months (Nov, Dec, Jan, Feb), intermediate months (Sept, Oct, Mar, Apr) and light months (May, Jun, Jul, Aug)] was performed. Median aPWV (8.9 m/s) and 33rd and 66th percentiles for PTH levels (47 pg/mL and 62 pg/mL, respectively) were calculated. Patients were then defined as having either higher or lower aPWV according to values above or below the median and above or below 12 m/s, the latter being the cut-off value proposed by the ESH/ESC guidelines for the management of hypertension\(^{36}\). Patients were grouped according to tertiles of PTH. Hence, a logistic regression model was performed with aPWV as a dependent categorical variable (higher vs lower) and tertiles of PTH, age, MAP and the heart rate as independent variables. Statistical significance was assumed if the null hypothesis could be rejected at \(p=0.05\).

Results
The characteristics of 150 postmenopausal
women with either 25-OH-vitamin D < 20 ng/mL or ≥ 20 ng/mL are summarized in Table 1. Briefly, patients with lower 25-OH-vitamin D levels had higher serum PTH levels (Panel B). 25-OH-vitamin D levels were negatively associated with aPWV (ρ = −0.39, p < 0.001). Further adjustment for the bone specific alkaline phosphatase, osteocalcin, albumin-corrected calcium levels, statin and antihypertensive therapies and month of examination did not influence the independent prediction of aPWV by LG-PTH levels (β = 0.20, p = 0.02).

In the logistic regression model with aPWV above the 50th percentile as a dependent variable, the third tertile of PTH levels was associated with a 3.0-fold increased probability of having higher aPWV (OR 3.0, 95% CI 1.1-7.9), irrespective of multivariate adjustment. When aPWV > 12 m/s was included as a categorical dependent variable, the third tertile of PTH levels was associated with a 5.4-fold increased probability of having higher aPWV (OR 5.4, 95% CI 1.1-28.2), independent of confounders.

**Discussion**

In the present cross-sectional sample of post-

**Table 1.** Characteristics of 150 postmenopausal women with either 25-OH-vitamin D < 20 ng/mL or ≥ 20 ng/mL.

<table>
<thead>
<tr>
<th></th>
<th>25-OH-vitamin D &lt; 20 ng/mL (n = 111)</th>
<th>25-OH-vitamin D ≥ 20 ng/mL (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 ± 9</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.6 ± 4.9</td>
<td>25.9 ± 4.9</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>139 ± 22</td>
<td>139 ± 16</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99 ± 14</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>Heart rate, n/min</td>
<td>69 ± 11</td>
<td>68 ± 11</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>82 ± 22</td>
<td>80 ± 24</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>92 ± 18</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>140 ± 34</td>
<td>147 ± 38</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>67 ± 16</td>
<td>67 ± 15</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>116 ± 48</td>
<td>117 ± 73</td>
</tr>
<tr>
<td>25-OH-vitamin D, ng/mL</td>
<td>11.9 ± 4.5</td>
<td>25.1 ± 3.2*</td>
</tr>
<tr>
<td>Parathyroid hormone, pg/mL</td>
<td>59.3 ± 18.0</td>
<td>50.6 ± 15.9*</td>
</tr>
<tr>
<td>Bone specific ALP, μg/L</td>
<td>14.6 ± 6.0</td>
<td>13.3 ± 4.1</td>
</tr>
<tr>
<td>Osteocalcin, ng/mL</td>
<td>13.7 ± 9.7</td>
<td>17.2 ± 10.8</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.2 ± 0.3</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Corrected calcium, mg/dL</td>
<td>9.3 ± 0.5</td>
<td>9.4 ± 0.5</td>
</tr>
<tr>
<td>Daily calcium intake, mg</td>
<td>669 ± 265</td>
<td>660 ± 256</td>
</tr>
<tr>
<td>Femur BMD, g/cm²</td>
<td>0.76 ± 0.16</td>
<td>0.79 ± 0.12</td>
</tr>
</tbody>
</table>

Values are the mean ± SD. *p < 0.05 for comparison between women with 25-OH-vitamin D < or ≥ 20 ng/mL. MAP, mean arterial pressure; GFR, glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALP, alkaline phosphatase; BMD, bone mineral density.
roidectomy has been associated with a significant reduction in aPWV\(^9,11\). Also, plasma PTH levels \(>50\) pg/mL accounted for 20% of the population-attributable risk proportion for cardiovascular mortality\(^40\). Hence, aortic stiffening, which is increasingly recognized as a surrogate endpoint for cardiovascular disease\(^1-6,41\), might represent one possible link between elevated PTH levels and cardiovascular risk. Although the association between PTH and aortic stiffness is often evident in patients with primary hyperparathyroidism\(^11,12\), it did not always emerge in the latter clinical setting\(^13,14\) or in patients with end-stage renal disease\(^15,16\). Furthermore, in community-dwelling elderly persons, the significant association between PTH and aPWV was attenuated and rendered no longer statistically significant after adjustment for several confounders\(^42\).

However, the association between PTH and aPWV has not yet been explored in the setting of postmenopausal women with normal kidney function and with 25-OH-vitamin D insufficiency, PTH levels were associated with aortic stiffness, irrespective of established cardiovascular risk factors and of factors involved in bone formation. A PTH level above the 66th percentile of this study (\(>62\) pg/mL) was associated with a three-fold to five-fold increased probability of having either a mild or even a severe increase in aortic stiffness.

Exposure to traditional cardiovascular risk factors, through degeneration of compliant elastin fibers, and the deposition of stiffer collagen, is considered a key cause of arterial stiffening\(^37-39\); however, additional factors might contribute to impaired aortic compliance.

An increase in aortic stiffness has been frequently observed in patients with primary hyperparathyroidism\(^9,12\) and the decline of PTH levels after parathyroidectomy has been associated with a significant reduction in aPWV\(^9,11\). Also, plasma PTH levels \(>50\) pg/mL accounted for 20% of the population-attributable risk proportion for cardiovascular mortality\(^40\). Hence, aortic stiffening, which is increasingly recognized as a surrogate endpoint for cardiovascular disease\(^1-6,41\), might represent one possible link between elevated PTH levels and cardiovascular risk. Although the association between PTH and aortic stiffness is often evident in patients with primary hyperparathyroidism\(^11,12\), it did not always emerge in the latter clinical setting\(^13,14\) or in patients with end-stage renal disease\(^15,16\). Furthermore, in community-dwelling elderly persons, the significant association between PTH and aPWV was attenuated and rendered no longer statistically significant after adjustment for several confounders\(^42\).

However, the association between PTH and aPWV has not yet been explored in the setting of postmenopausal women with normal kidney function and with 25-OH-vitamin D insufficiency. Hence, the discordance between the results of the present study and those observed in other clinical settings might be simply justified by the different study populations. Also, the small sample size in the study by Kosch et al.\(^13\) and, in the study by Tordjman et al.\(^14\), the presence of multiple possible covariates (i.e. diabetes, thyroid diseases, cardiovascular disease, calcium and vitamin D supplements, other than hypertension and dyslipidemia) of both PTH and aPWV might have confounded the association between PTH levels and aor-

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Fig. 1. Aortic pulse wave velocity (aPWV) in patients with either 25-OH-vitamin D <20 ng/mL or \(\geq20\) ng/mL. Panel A, unadjusted means. Panel B, adjusted for PTH levels. Values inside the bars are the means \(\pm\) SE.

Fig. 2. Correlation between log-transformed PTH (LG-PTH) levels and aortic pulse wave velocity (aPWV).
tic stiffness. Moreover, it should be kept in mind that several factors, other than PTH, are established determinants of arterial stiffening in patients with end-stage renal disease and include malnutrition, chronic volume overload, dialysated calcium and others. Importantly, in the present study, inclusion of patients with a normal glomerular filtration rate allowed us to exclude most of these confounders.

Although the observational design of the study does not allow us to reach conclusions on the mechanism underlying the association between PTH and aPWV, there is some speculative evidence supporting this association. First, PTH has been implicated in vascular calcification in the setting of end-stage renal disease, and it is a well-recognized contributor to arterial stiffening. Second, PTH appears to also increase total collagen synthesis and reorganized collagen in cultured vascular smooth muscle cells, although PTH also prevented bovine vascular smooth muscle cell calcification in vitro. Third, higher PTH levels are associated with established covariates of arterial stiffness, such as hypertension, obesity and and renal dysfunction; however, the fact that PTH remained significantly associated with aortic stiffness in all multivariable models suggests that confounding by these factors is not the sole explanation for our findings. We cannot rule out the possibility of residual confounding or confounding by unmeasured factors.

A prospective association between reduced levels of 25-OH-vitamin D and cardiovascular risk has been observed; however, in a study of African Americans with type 2 diabetes mellitus, 25-OH-vitamin D levels correlated with increased calcified plaque in the aorta and carotids. Thus, concern has been expressed that vitamin D could potentially accelerate vascular disease.

We found an inverse, albeit weak, association between 25-OH-vitamin D and aPWV that was, however, fully dependent on serum PTH levels. To the best of our knowledge, only two studies have specifically explored the association between vitamin D status and aortic stiffness. In end-stage renal disease, London et al. found that levels of 25-OH-vitamin D were negatively correlated with aPWV, irrespective of age and blood pressure levels. Again, in the sample of patients on hemodialysis, the association between PTH and aPWV was not detected; hence, the correlation between 25-OH-vitamin D and aPWV was not fairly adjusted for PTH levels. In the recent study by Al Mheid et al., levels of 25-OH-vitamin D were negatively correlated with aPWV, independent of multiple confounders. However, in that study of a mixed population of healthy men and women, the correlation between 25-OH-vitamin D and aPWV was not adjusted for PTH levels. With these differences in the study populations and in the statistical approach between our study and that of London et al. and Al Mheid et al., there was conceivably a difference in the degree of correlation between 25-OH-vitamin D and aPWV.

As expected, we also found a significant negative association between 25-OH-vitamin D and PTH levels, which is explained by the fact that PTH secretion increases in response to low vitamin D levels. Hence, we suggest that in postmenopausal women with reduced 25-OH-vitamin D levels and normal renal function, PTH elevation due to 25-OH-vitamin D insufficiency might contribute to aortic stiffening.

The potential limitations of our study should be noted. The study was restricted to a limited number of postmenopausal women with reduced 25-OH-vitamin D levels; thus, the results may not be applicable to younger women, men, or subjects with normal 25-OH-vitamin D levels. Also, from a statistical perspective, the inclusion of patients with 25-OH-vitamin D insufficiency with a relatively narrow range of 25-OH-vitamin D levels might have precluded the demonstration of a stronger association between vitamin D and aPWV; hence, studies in subjects with a wider range of vitamin D levels are awaited to further deepen the understanding of the relationship between 25-OH-vitamin D and arterial stiffness. Moreover, the observational design of the study does not allow us to reach conclusions on the mechanism underlying the observed statistical association between PTH and aPWV; independent PTH prediction of aPWV needs to be validated in prospective and interventional studies.

**Conclusion**

In postmenopausal women with reduced 25-OH-vitamin D levels, increases in PTH levels, possibly sustained by reduced 25-OH-vitamin D levels, are associated with mild to severe aortic stiffening irrespective of traditional cardiovascular risk factors and markers of bone formation. The inverse, albeit weak, association between 25-OH-vitamin D and aPWV was dependent on serum PTH levels.

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Conflict of Interest

No.

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