Plasma–Parathyroid Hormone Is Associated With Subclinical and Clinical Atherosclerotic Disease in 2 Community-Based Cohorts

Emil Hagström, Karl Michaëlsson, Håkan Melhus, Thomas Hansen, Håkan Ahlström, Lars Johansson, Erik Ingelsson, Johan Sundström, Lars Lind, Johan Ärnlöv

Objective—Cardiovascular risk factors have different impact on different arterial territories. Diseases with elevated circulating parathyroid hormone (PTH) such as primary hyperparathyroidism and chronic renal failure have been shown to be associated with an increased risk of cardiovascular disease, predominantly heart or cerebrovascular diseases. However, data on the associations between circulating PTH and peripheral atherosclerosis are limited.

Approach and Results—Two prospective, community-based studies were used. In 306 men and women, who were 70 years old, from the Prospective investigation of the vasculature in Uppsala seniors (PIVUS) study, cross-sectional relations between PTH and atherosclerotic burden assessed by whole-body magnetic resonance angiography were investigated. In 998 men, who were 71 years old, from the Uppsala longitudinal study of adult men (ULSAM) study, the association between PTH concentration and risk of subsequent nonfatal atherosclerotic disease (excluding coronary or cerebrovascular disease) was investigated. Adjusting for established vascular risk factors, PTH was associated with burden of atherosclerosis (increase in total atherosclerotic score per SD PTH increase: 0.04, 0.003–0.08; P=0.03) in the PIVUS study. During follow-up in the ULSAM study (median 16.7 years), 89 men were diagnosed with nonfatal atherosclerotic disease. In Cox-regression analyses adjusting for established vascular risk factors and mineral metabolism, higher PTH was associated with an increased risk of nonfatal atherosclerotic disease (hazard ratio for 1 SD increase of PTH: 1.55, 1.33–1.88; P<0.0001). Results were similar when including fatal atherosclerotic disease in the outcome.

Conclusions—In 2 independent community-based cohorts, PTH was associated to the degree of atherosclerosis and risk of clinically overt atherosclerotic disease, respectively. Our data confirm and extend previous studies supporting a role for PTH in the development of atherosclerotic disease. (Arterioscler Thromb Vasc Biol. 2014;34:1567-1573.)

Key Words: atherosclerosis ■ magnetic resonance angiography ■ parathyroid hormone ■ population

Patients with increased levels of parathyroid hormone (PTH), resulting from primary hyperparathyroidism or chronic kidney disease (CKD) with secondary hyperparathyroidism, have high risk for cardiovascular diseases (CVDs) and death.

See accompanying editorial on page 1333

Multiple lines of evidence from both experimental and clinical studies suggest that PTH may be involved in the development of subclinical and overt vascular diseases with endothelial dysfunction, increased vascular stiffness, hypertension, and precerebral artery atherosclerosis. Moreover, an elevated risk of overt CVD and death has been related to increasing levels of PTH, a risk that seems to be present already within normal ranges. There are also reports of associations between other variables of the mineral metabolism such as increasing levels of phosphate or calcium and low levels of vitamin D and vascular diseases. Yet to date, our knowledge on the association of circulating PTH and the risk for clinical and subclinical atherosclerotic vascular disease in the community is limited. This may be important because different cardiovascular risk factors have different impact in different arterial territories.

We hypothesized that higher PTH is a causal factor for atherosclerosis in both peripheral and central arteries. Accordingly, we aimed to investigate the cross-sectional association between PTH and atherosclerotic burden assessed by whole-body magnetic resonance imaging (MRI) angiography in a community-based cohort of elderly men and women. Whole-body angiography by MRI is a novel way of assessing the total atherosclerotic burden in the vasculature outside the heart and brain, without the use of ionizing radiation.
results from the whole-body MRI angiography are achieved in one single session and are in agreement with the established gold standard method based on conventional angiography and are associated with cardiovascular outcomes. As a second step, we studied the prospective association between PTH and the risk of peripheral and large vessel atherosclerotic disease in an independent community-based sample of elderly men.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

Baseline Characteristics

Baseline characteristics of both cohorts are shown in Tables 1 and 2. Individuals in ULSAM and PIVUS had similar age (70 and 71) and body mass index (26.3 and 27), with a smoking prevalence of 20% in the former and 9% the latter.

PTH Levels and Vascular Disease

PIVUS

Higher levels of PTH were associated with increasing atherosclerotic burden according to whole-body magnetic resonance angiography (WBMRA) in linear regressions (Model D: 1 SD increase in PTH was associated with 0.041 higher category of total atherosclerosis score, 95% confidence intervals [CI] 0.002–0.08], P=0.038). This association was present in all models, adjusting for vascular risk factors and for mineral metabolism (Table 3). The relation between PTH and total atherosclerosis score showed no deviation from linearity when introducing a quadratic term of PTH (P=0.47), and a regression spline graph also suggests that there seems to be a linear increase in hazard for atherosclerosis (Figures 2 and 3) and the combination of disease and death because of atherosclerosis, with increasing PTH levels. In secondary analyses, a 1 SD increase in plasma-PTH was associated with a 32% to 46% higher risk for fatal and nonfatal atherosclerotic disease (Models A–D, Table 5).

Population-Attributable Risk Proportion

Elevated PTH (as defined by the quartile 4 versus 1–3 cutoff, plasma-PTH>5.27 pmol/L) accounted for 11.6% (95% CI [4–16]) of the population-attributable risk proportion (PAR) for nonfatal atherosclerotic disease. The PAR values for the other cardiovascular risk factors in model D are given in Table 6 (corresponding Cox proportional hazard ratios for vascular risk-factors are presented in Table 1 in the online-only Data Supplement). PTH and smoking were the only variables associated with vascular atherosclerotic disease.

Sensitivity Analysis

Furthermore, in model D, in individuals without CKD (1 SD increase of PTH: HR, 1.55; 95% CI [1.10–2.19]; P=0.012) or hypercalcemia (HR for 1 SD increase of PTH: 1.52; 95% CI [1.25–1.85]; P<0.0001), respectively, the associations between PTH and vascular disease were similar in all models, as well as when adding C-reactive protein, cystatin C, N-terminal proB-type natriuretic peptide, and troponin I to the model D for the whole cohort (1 SD increase of PTH; HR, 1.51; 95% CI [1.24–1.85]; P<0.0001).

Table 1. Study Characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>PIVUS</th>
<th>ULSAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>306</td>
<td>998</td>
</tr>
<tr>
<td>Study design</td>
<td>Cross-sectional</td>
<td>Longitudinal</td>
</tr>
<tr>
<td>Follow-up time</td>
<td>…</td>
<td>16.7 y (range 0.01–20.4 y)</td>
</tr>
<tr>
<td>Exposure</td>
<td>P-PTH</td>
<td>P-PTH</td>
</tr>
<tr>
<td>PTH method</td>
<td>Immulite 2000 Intact PTH Assay (Diagnostic Products Corporation, Los Angeles, CA)</td>
<td>Immulite 2500 Intact PTH Assay (Diagnostics Product Corporation, Los Angeles, CA)</td>
</tr>
<tr>
<td>Outcome</td>
<td>TAS</td>
<td>Nonfatal noncardiac noncerebral atherosclerotic disease</td>
</tr>
<tr>
<td>Outcome assessment</td>
<td>WBMRA</td>
<td>The Swedish Hospital Discharge Register</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>Multivariable linear regression</td>
<td>Cox proportional hazard regression</td>
</tr>
</tbody>
</table>

MRI indicates magnetic resonance imaging; P-PTH, plasma–parathyroid hormone; PIVUS, Prospective investigation of the vasculature in Uppsala seniors; TAS, total atherosclerosis score; ULSAM, Uppsala longitudinal study of adult men; and WBMRA, whole-body magnetic resonance angiography.

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>PIVUS</td>
<td>Prospective investigation of the vasculature in Uppsala seniors</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>ULSAM</td>
<td>Uppsala longitudinal study of adult men</td>
</tr>
<tr>
<td>WBMRA</td>
<td>whole-body magnetic resonance angiography</td>
</tr>
</tbody>
</table>
### Discussion

**Principal Findings**

In the present study, increasing levels of the hormone PTH, a key regulator of the mineral metabolism, were associated with the level of atherosclerotic burden as assessed by WBMRA in a community-based cohort of the elderly. Moreover, adding further support and additional clinical relevance of these findings, higher PTH was associated with an increased risk for clinically overt nonfatal atherosclerotic disease, independently of both established risk factors for CVDs and of mineral metabolism in an independent community-based cohort of elderly men. Elevated plasma levels of PTH accounted for ≈12% of the population-attributable risk proportion for clinical atherosclerotic disease, on par in risk with smoking. These findings indicate that elevated PTH could have substantial public health implications.

### Comparison With Previous Studies

The present results are in accordance with previous experimental and clinical studies reporting vascular abnormalities with increasing levels of PTH, but no previous study has presented data on PTH in relation to atherosclerosis in different arterial territories as assessed by whole-body angiography. Furthermore, the findings that PTH concentrations predict vascular diseases and death have been thoroughly described in studies of patients with mineral metabolism diseases such as primary and secondary hyperparathyroidism as well as CKD. Also in the community-based setting, higher PTH have been shown to be associated with an increased risk for cardiovascular mortality, a composite endpoint predominantly consisting of death attributable to coronary heart disease and cerebrovascular disease, or the risk for heart failure. The association between PTH and the risk for peripheral or large vessel atherosclerotic disease has not been reported previously in the community-based setting.

### Potential Mechanisms

Several mechanisms may explain the relationship between PTH and atherosclerotic disease in this study. First, PTH has been related to atherogenesis via vascular calcification and vascular remodeling both via direct PTH receptor interaction on the vessel as well as indirectly via inflammation and vascular dysfunction. Second, PTH seems to be involved with diseases of the myocardium via induction of left ventricular hypertrophy, congestive heart failure, cardiac calcification and fibrosis, detrimental states that all have secondary negative effects on the vasculature. Third, higher PTH is associated with both established cardiovascular risk factors and more recently described risk factors such as markers of inflammation, renal dysfunction, and cardiac pathology. The fact that PTH was consistently associated with atherosclerotic disease and death in all multivariable models suggests that confounding by these other factors is not the sole explanation for our findings. Moreover, the results from the WBMRA provide further support of previous data of PTH being associated to subclinical and clinical atherosclerotic diseases.

In this study setting, it is not possible to fully investigate the separate contribution by PTH on the development of atherosclerosis.

### Table 2. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>ULSAM</th>
<th>PIVUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>998</td>
<td>306</td>
</tr>
<tr>
<td>Age, y</td>
<td>71.0±0.6</td>
<td>70.1±0.2</td>
</tr>
<tr>
<td>Body mass index, kg/m^2</td>
<td>26.3±3.4</td>
<td>27.0±3.9</td>
</tr>
<tr>
<td>S-Cholesterol, mmol/L</td>
<td>5.8±1.0</td>
<td>5.5±1.0</td>
</tr>
<tr>
<td>S-Low-density lipoprotein, mmol/L</td>
<td>3.9±0.9</td>
<td>3.4±0.8</td>
</tr>
<tr>
<td>S-High density lipoprotein, mmol/L</td>
<td>1.3±0.4</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>147±19</td>
<td>149±21</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>84±9</td>
<td>79±10</td>
</tr>
<tr>
<td>Intact P–PTH, pmol/L</td>
<td>4.0 (3.0, 5.3)</td>
<td>5.0±2.3</td>
</tr>
<tr>
<td>S-Calculator, mmol/L</td>
<td>2.3±0.1</td>
<td>2.3 (2.3, 2.4)</td>
</tr>
<tr>
<td>S-Aluminin, g/L</td>
<td>43.1±2.6</td>
<td>40.6±2.8</td>
</tr>
<tr>
<td>S-Phosphate, mmol/L</td>
<td>0.95 (0.85, 1.1)</td>
<td>1.1±0.16</td>
</tr>
<tr>
<td>P–25–OH vitamin D, nmol/L</td>
<td>68±24</td>
<td>59±20</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL/min per 1.73 m^2</td>
<td>61±14</td>
<td>83±21</td>
</tr>
<tr>
<td>S-C-reactive protein, mg/L</td>
<td>1.9 (0.94, 3.9)</td>
<td>2.9±3.7</td>
</tr>
<tr>
<td>P–NT-pro brain natriuretic peptide, ng/L</td>
<td>108 (60, 224)</td>
<td>101 (61, 174)</td>
</tr>
<tr>
<td>Smoker</td>
<td>245 (20)</td>
<td>36 (9)</td>
</tr>
<tr>
<td>Diabetes mellitus prevalence</td>
<td>131 (11)</td>
<td>27 (6.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>909 (74)</td>
<td>291 (72)</td>
</tr>
<tr>
<td>Antihypertensive treatment</td>
<td>295 (34)</td>
<td>113 (32)</td>
</tr>
<tr>
<td>Lipid-lowering treatment</td>
<td>107 (8.8)</td>
<td>56 (14)</td>
</tr>
</tbody>
</table>

Values are means (±SD), median (interquartile ranges), and n (%) for categorical variables. Conversion factor for plasma–parathyroid hormone (P–PTH; from pmol/L to pg/mL): [(pmol/L)/0.1053]. PIVUS indicates Prospective investigation of the vasculature in Uppsala seniors; and ULSAM, Uppsala longitudinal study of adult men.

### Table 3. PIVUS. Multiple Linear Regression Analysis of Plasma–PTH to Total Atherosclerotic Burden (n=306) Analyzed With Whole-Body Magnetic Resonance Angiography

<table>
<thead>
<tr>
<th></th>
<th>Model A Unadjusted</th>
<th>Model B CVD Risk Factors</th>
<th>Model C Mineral Metabolism Variables</th>
<th>Model D CVD Risk Factors+Mineral Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SD increase</td>
<td>0.040 (0.001–0.08)*</td>
<td>0.042 (0.003–0.08)*</td>
<td>0.04 (0.001–0.08)*</td>
<td>0.041 (0.002–0.08)*</td>
</tr>
</tbody>
</table>

Data are linear regression coefficients (95% confidence intervals) for 1 SD increase of plasma–parathyroid hormone (P–PTH) in model A, unadjusted; B, adjusted for established risk factors for vascular disease (hypertension, diabetes mellitus, smoking, body mass index, hypercholesterolemia); C, adjusted for factors associated with mineral metabolism (S-calcium, S-phosphate, S-aluminin, P–25–OH vitamin D, glomerular filtration rate, blood draw season [winter, summer]); model D, combination of multivariable models B and C. CVD indicates cardiovascular disease; and PIVUS, Prospective investigation of the vasculature in Uppsal seniors. *P<0.05.
Atherosclerosis in relation to other mineral metabolism variables because the levels are highly dependent on each other. Furthermore, all variables in the mineral metabolism have been associated with the development of CVDs, ranging from subclinical endothelial dysfunction to myocardial infarction and cardiovascular death.11,12,23 However, in this study, the association between PTH and atherosclerosis persisted after adjustments for vitamin D status (serum 25-hydroxyvitamin D levels determined by the gold standard method), time of year for biochemical analysis, and other mineral metabolism variables, in conjunction with persistent results associating PTH and atherosclerotic in an independent cohort with another modality assessing degree of atherosclerosis.

**Clinical Implications**

Because vascular diseases in the community are common and often have negative impact on the individual as well as being a burden for society, there is a great need to explore underlying mechanisms.32,33 As different cardiovascular risk factors have different impact on the prediction of atherosclerotic disease in different parts of the vasculature, it was important to also investigate the association between PTH and atherosclerosis outside the heart and brain. In patients who are treated or cured from disorders of mineral metabolism, cardiovascular abnormalities, such as hypertension, vascular dementia, and vascular calcifications, may improve or not progress further.34–36 If future studies could show that PTH lowering leads to a reduced atherosclerotic burden, there is potentially a large public health implication because PTH lowering could fairly be managed by vitamin D fortification of the diet or by supplementation. Although PTH was associated to atherosclerotic events and to imaging evidence of atherosclerosis in the present study, the use of measuring PTH in a clinical setting for risk assessment remains to be evaluated.

**Figure 1.** Nelson–Aalen plot of cumulative incidence rate of atherosclerotic disease (nonfatal) in the whole sample by 2 groups (parathyroid hormone [PTH] quartiles 1 to 3 [solid line] vs quartile 4 [dashed line]) in the Uppsala longitudinal study of adult men cohort.

**Figure 2.** Association between plasma–parathyroid hormone (P-PTH) and incidence of atherosclerotic disease in the Uppsala longitudinal study of adult men cohort. Horizontal curves show estimated hazard ratios (with confidence limits) for atherosclerotic disease in relation to P-PTH as a function of penalized regression splines. Q indicates quartile; vertical lines indicate quartile division.
Strengths and Limitations
Strengths include the use of 2 large, homogenous, community-based populations, with detailed characterization of potential confounders, and in ULSAM a long follow-up. Furthermore, the association between PTH and atherosclerosis was strengthened by WBMRA associating PTH and manifest atherosclerotic disease.

Table 5. ULSAM. Relations of Plasma-PTH to Atherosclerotic Disease and Death

<table>
<thead>
<tr>
<th>Model</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 SD increase</td>
<td>1.32 (1.14–1.54)*</td>
<td>1.42 (1.21–1.66)*</td>
<td>1.31 (1.14–1.52)*</td>
<td>1.46 (1.24–1.71)*</td>
</tr>
<tr>
<td>Multicategory models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (&lt;2.96 pmol/L)</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Q2 (2.95–3.99 pmol/L)</td>
<td>1.04 (0.69–1.57)</td>
<td>1.07 (0.65–1.78)</td>
<td>1.14 (0.69–1.89)</td>
<td>1.17 (0.70–1.94)</td>
</tr>
<tr>
<td>Q3 (3.99–5.27 pmol/L)</td>
<td>1.08 (0.68–1.77)</td>
<td>1.11 (0.69–1.86)</td>
<td>1.19 (0.72–1.98)</td>
<td>1.22 (0.73–2.03)</td>
</tr>
<tr>
<td>Q4 (&gt;5.27 pmol/L)</td>
<td>1.47 (0.91–2.35)</td>
<td>1.73 (1.06–2.74)†</td>
<td>1.69 (1.05–2.74)†</td>
<td>1.93 (1.18–3.18)‡</td>
</tr>
<tr>
<td>Threshold models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q4 vs Q1–3 (&gt;5.27 pmol/L)</td>
<td>1.42 (0.98–2.04)10.16</td>
<td>1.62 (1.11–2.35)†</td>
<td>1.52 (1.05–2.21)†</td>
<td>1.59 (1.09–2.32)†</td>
</tr>
</tbody>
</table>

Data are Cox proportional hazards ratios (95% confidence intervals) for a 1 SD log parathyroid hormone (PTH) increase in; model A, unadjusted; B, adjusted for established risk factors for vascular disease (hypertension, diabetes mellitus, smoking, body mass index, hypercholesterolemia); C, adjusted for factors associated with mineral metabolism (S-calcium, S-phosphate, S-albumin, P-25-OH vitamin D, glomerular filtration rate, blood draw season [winter, summer]); model D, combination of multivariable models B and C. CVD indicates cardiovascular disease; Q, quartile; and ULSAM, Uppsala longitudinal study of adult men.

*P<0.001; †P<0.05; ‡P<0.01.
atherosclerosis. Limitations in the PIVUS study include that MR investigations were only performed in a random subset of the individuals. The total atherosclerotic burden is partly based on a visual assessment and manual measurement of degree of vascular pathology that may introduce error. However, the relation between atherosclerosis detected on WBMRA and vascular outcomes has been described previously, and results from WBMRA investigations are in agreement with digital subtraction angiography, the gold standard method for assessing degree of atherosclerotic burden. Also, the manual assessment was blinded to measures of PTH. Increased support for the validity of our findings are previous studies strongly relating imaging evidence of burden of atherosclerosis to increased risk of disease and death from atherosclerotic diseases.

Limitations in the ULSAM study include the unknown generalizability to women and for both cohorts, to other age- and ethnic groups because we only examined individuals of the same age and ethnic background. Because of the large number of covariates in the fully adjusted model and risk of overfitting, we applied a propensity score to the model to decrease the risk of introducing random errors.

In both cohorts, levels of plasma-PTH and other variables included in the models were measured only at the baseline examination, and it is not known how well a single measurement reflects the PTH levels during the follow-up. Yet, any potential bias by variations in PTH levels over time would most likely conservatively bias our risk estimates because PTH increases with ageing. Albeit this uncertainty, the data clearly show that a spot sample of plasma-PTH levels is an independent predictor for both clinical and subclinical atherosclerotic disease. Further studies are needed to properly address the issue of identifying clinically relevant thresholds of circulating PTH for risk prediction purposes.

Conclusions

Increasing levels of PTH was associated with degree of atherosclerotic burden assessed by WBMRA and predicted morbidity and mortality of peripheral and large vessel atherosclerotic disease, in 2 independent cohorts in the community. Our data add to the notion that PTH may play an important role for the development of atherosclerosis in both peripheral and central arteries.

Sources of Funding

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Disclosures

Drs Lind and Ahlström report grant support from AstraZeneca. The other authors report no conflicts.

References


### Significance

Atherosclerotic vascular disease is a major cause of morbidity and mortality with a great need for disentangling underlying mechanisms. Patients with elevated levels of parathyroid hormone (PTH), a key hormone of the mineral metabolism, such as in chronic kidney disease, are at high risk for vascular morbidity and mortality. Evidence from experimental and clinical studies suggests that PTH may be involved in the pathological process leading to vascular disease. To date, the prospective association between PTH and incident vascular disease in the general population is sparse. This study describes a robust association between increasing levels of PTH and degree of atherosclerotic burden assessed by whole-body magnetic resonance angiography and prediction of morbidity and mortality of peripheral and large vessel atherosclerotic disease in 2 independent community-based cohorts. The study indicates that PTH may be a marker for development of atherosclerotic disease.
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Supplementary table I.

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Highest quartile of PTH</td>
<td>1.87*</td>
<td>1.16-2.99</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.18</td>
<td>0.97-1.39</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.43</td>
<td>0.88-2.32</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.50</td>
<td>0.85-2.7</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.66</td>
<td>0.92-3.3</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.1*</td>
<td>1.3-3.34</td>
</tr>
</tbody>
</table>

Data are Cox proportional hazards ratios (95% CI) for established risk factors and PTH. Highest quartile of PTH > 5.27 pmol/L. Diabetes mellitus was defined as fasting plasma glucose ≥7.0 mmol/l, two hours post-load glucose levels ≥11.1 mmol/l, or the use of oral hypoglycaemic agents or insulin. Obesity was defined as a body mass index ≥30 kg/m², calculated as (weight (kg)/height² (m)). Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or use of antihypertensive medication. Hypercholesterolaemia was defined as total serum cholesterol ≥5 (mmol/L) or the use of pharmacological treatment for dyslipidaemia. Smoking status: current smoking versus no-smoking. *Variables with significant association to outcome.
Methods

Study Sample
The study is based on two longitudinal community-based cohorts, the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)\(^1\) and the Uppsala Longitudinal Study of Adult Men (ULSAM).\(^2\) All participants gave written informed consent and the Ethics Committee of Uppsala University approved the studies.

PIVUS
All men and women aged 70 years old living in Uppsala, Sweden, were eligible for inclusion in this study and 1,016 subjects accepted a written invitation (participant rate 50.1 \%).\(^1\) Of these, 306 subjects (145 women, 161 men) were randomly recruited and were investigated with whole body magnetic resonance angiography (WBMRA).\(^3\) Age, PTH and all included covariates were similar in individuals with or without investigation with WBMRA. Exclusion criteria for the WBMRA examination were pacemaker, valvular prostheses, intracranial clips, and claustrophobia.

ULSAM
The Uppsala Longitudinal Study of Adult Men (ULSAM) was initiated in 1970. All 50-year-old men (n=2322) born in 1920 to 1924 and living in Uppsala, Sweden, were invited to a health survey focusing on identifying cardiovascular risk factors.\(^2\),\(^4\) A second examination cycle was performed when the participants were approximately 60 years old.

The present analysis was based on the third examination cycle when the participants were approximately 71 years old and included 998 men (82 % of the investigated cohort of 1,221 individuals) after exclusion of individuals with missing data, primarily due to missing PTH measurements (n=223). For most variables there were a few individuals with missing data on covariates (phosphate having the highest missing value ratio with 3.6 \% missing). In the present cohort incidence rate of atherosclerotic disease per 1000 person-years at risk was 5.4 in both included and excluded participants. Analyses were also performed in individuals without renal dysfunction (glomerular filtration rate $\geq 60$ mL/min/1.73m\(^2\), n= 746) and in individuals without hypercalcemia (n=946).

Baseline Examinations
In both cohorts, venous blood for biochemical analyses was drawn after an overnight fast at the baseline investigations for the study and analyses has been described previously.\(^1\),\(^4\) Height, weight, body mass index (weight (kg)/height\(^2\) (m), BMI), and supine systolic and diastolic blood pressures were measured under standardized conditions. Hypertension was defined as systolic blood pressure $\geq 140$ mmHg, diastolic blood pressure $\geq 90$ mm Hg, or use of antihypertensive medication, diabetes mellitus as P-glucose $\geq 7.0$ mmol/L, or use of oral hypoglycemic agents or insulin, hypercholesterolemia
as S-cholesterol > 5 mmol/L, LDL-cholesterol >3 mmol/L or use of lipid-lowering treatment and renal dysfunction as glomerular filtration rate < 60 mL/min/1.73 m$^2$. Smoking status (never, former) was obtained from questionnaires.

**PIVUS**

P-PTH was measured with an immunoassay (Table 1). S-calcium (normal range 2.20-2.50 mmol/L) and S-albumin (normal range 37-48 g/L) were measured spectrophotometrically. Serum cystatin C, creatinine and phosphate measurements were performed on an Architect Ci8200 analyzer (Abbott Laboratories, Abbott Park, IL, USA). Estimated glomerular filtration rate (GFR) was derived from cystatin C, by the formula $\text{eGFR}_{\text{CystC}} = 79.901 \times \text{CystC}^{-1.439}$. P-25-OH vitamin D was analyzed with a Liason 25(OH)D$_3$ assay in a Liason analyzer (DiaSorin Inc., Saluggia, Italy).

**ULSAM**

P-PTH was measured with an immunoassay (Table 1). S-calcium [normal range 2.2 to 2.6 mmol/L], albumin and phosphate were measured with spectrophotometry. The instruments used for biochemical analysis were Hitachi 717 or 911 (Hitachi, Japan). Plasma 25-OH vitamin D was determined with high-performance liquid chromatography (HPLC) together with atmospheric pressure chemical ionization (APCI) and mass spectrometric detector (MS) at Vitas A/S, Oslo, Norway (www.vitas.no) using a HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto CA, USA). Serum cystatin C was measured by latex enhanced reagent (N Latex Cystatin C, Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec analyzer (Dade Behring). Glomerular filtration rate was calculated from serum cystatin C results in mg/L by the formula $y = 77.24x^{-1.263}$ which has been shown to be closely correlated with iohexol clearance.

**Magnetic resonance imaging**

*Image acquisition*

The whole body magnetic resonance angiography (WBMRA) examination was performed on a 1.5 Tesla MRI system (Gyrosan Intera, Philips Medical Systems, Best, Netherlands), described previously. Three dimensional RF-spoiled, T1-weighted gradient echo acquisition was performed prior to and after injection of 40 ml of Gadodiamide. The investigations were performed 3-22 months (mean 16 months) after the study baseline.

*Image analysis*

The arterial tree was divided into 26 vessel segments and allocated to 1 of 5 groups: no stenosis, 1% to 49% reduction in lumen diameter, 50% to 99%
reduction in lumen diameter, occlusion, or aneurysm. The right and left sides were evaluated separately. Each segment was graded only according to the most severe stenosis. Significant atherosclerotic abnormality was defined as a reduction of the vessel diameter by 50% or more, or occlusion. A radiologist blinded of baseline data evaluated all WBMRA examinations. The 26 vessel segments were categorized into 5 territories: 1, the carotids, including the internal carotid artery and common carotid artery; 2, the aorta, including both the thoracic and abdominal part; 3, the renal arteries; 4, the pelvic/upper limbs, including the common iliac artery, external iliac artery, common femoral artery, superficial femoral artery, and popliteal artery; and 5, the lower legs, including the tibio-peroneal trunk, anterior tibial artery, peroneal artery, and posterior tibial artery. Cerebral or cardiac arteries were not included in this categorization.

To obtain a comparable graded number reflecting the atherosclerosis in each territory, an atherosclerotic score was calculated for each territory, total atherosclerosis score (TAS). A normal vessel segment received zero points, <50% stenosis was given 1 point and ≥50% stenosis, including occlusions, was given 2 points (Figure 3 a-c). The points for the vessel segments in a territory were summarized and divided by the maximum sum that would be achieved if all included segments had >50% stenosis or occlusion. The ratio was thereafter multiplied by 100. Hence, each territory could attain a maximum atherosclerotic score of 100. Vessel segments not possible to evaluate were excluded from the calculations. The reproducibility and reliability of the scoring system have previously been investigated. WBMRA detected atherosclerosis and vascular outcomes has been described previously and results from WBMRA investigations are in agreement with digital subtraction angiography, the gold standard method for assessing atherosclerotic burden.

Outcomes

**ULSAM**

The Swedish Hospital Discharge Register was used to define the primary endpoint: non-fatal non-cardiac non-cerebral atherosclerotic disease such as verified atherosclerosis, occlusions or stenosis in peripheral, central and precerebral arteries such as; thoracic and abdominal aorta, renal, extremity and precerebral arteries.

The diseases captured include stenosis or occlusions of carotid or vertebral arteries, claudicatio of the upper and lower extremities, atherosclerosis of the aorta, extremity atherosclerosis with gangrene, renal artery stenosis or occlusions [ICD-10] code I70 and I73 and I65 and [ICD-9] code 440-442 and 433).

The Swedish Cause of Death Register was used to define a pre-specified secondary sensitivity endpoint consisted of non-cardiac and non-cerebral composite of atherosclerotic and vascular disease and non-cardiac and non-cerebral atherosclerotic and vascular death ([ICD-10] code I70 and I73 and I65, [ICD-9] code 440-442 and 433). Coronary heart disease (such as myocardial infarction, stable coronary heart disease) and cerebrovascular
disease (ischemic or hemorrhagic stroke, transient ischemic attack, small or large vessel cerebral disease) were not included as endpoints in any analyses. The individuals were being at risk until censored at the date of the first primary event during follow-up.

Statistical Analyses

**PIVUS**

Logarithmic transformation (natural logarithm) was performed to achieve normal distribution for skewed variables (calcium, NT-proBNP). The relation between PTH and total atherosclerosis score (TAS) was investigated with multivariable linear regression in the whole cohort, using the following four models:

A. unadjusted
B. adjusted for vascular risk factors [hypertension [dichotomous], diabetes mellitus [dichotomous], smoking (never, current) [dichotomous], BMI [continuous], hypercholesterolemia [dichotomous]];  
C. adjusted for mineral metabolism [S-calcium, S-phosphate, S-albumin, P-25-OH vitamin D, glomerular filtration rate [all continuous], blood draw season (winter, summer)];
D. adjusted for vascular risk factors and mineral metabolism variables (model B+C)

PTH was modeled as a continuous standardized variable.

**ULSAM**

Logarithmic transformation (natural logarithm) was performed for skewed variables in all analyses (PTH, phosphate, CRP, NT-proBNP). The relation of log PTH (below only denoted PTH) to atherosclerotic vascular disease was investigated in the primary analysis using Cox proportional hazard regression in the whole cohort, using the same multivariable models as in the PIVUS cohort (models A-D).

Due to the large number of covariates in model D we also used propensity scores to support the validity of the findings in this model. The individual propensity scores, defined as the conditional probability of having PTH > 5.27 pmol/L or ≤ 5.27 pmol/L (quartile 4 versus quartiles 1 to 3), were estimated by multivariable logistic regression models. Multiple imputation methods were used to account for the potential influence of missing data (with the ice and mim commands in STATA) assuming that the data were missing at random. The primary exposure and outcome variables were not imputed. Ten imputed datasets were used.

In our primary analyses, investigating atherosclerotic disease, PTH was modeled as a continuous standardized variable. We also used multi-category models comparing risk in PTH quartiles 2 to 4 with that in quartile 1 (lowest) and threshold models (modeled as quartile 4 versus quartiles 1 to 3 [PTH > 5.27 pmol/L]).

In secondary analyses, the relation between PTH and a composite endpoint of fatal and non-fatal atherosclerotic vascular disease was analyzed. Also the association between PTH and atherosclerotic vascular disease and death in individuals without chronic kidney disease (defined as glomerular
filtration rate < 60 mL/min/1.73 m²) was investigated. Furthermore, newer risk factors (CRP, cystatin C, NT-proBNP and troponin I) were also added to the models investigating the relation between PTH and vascular disease and death.

Test of linearity was assessed by adding a quadratic term of PTH to multivariable model B. To gain additional insights into potential nonlinearity of the association between PTH and vascular disease, we examined the Cox regression model using penalized splines with three degrees of freedom (knots at 10th [2.17 pmol/L], 50th [3.99 pmol/L], and 90th percentile [6.76 pmol/L] of PTH). The proportional hazards assumptions for the models were assessed by comparing Nelson-Aalen plots and confirmed formally by Schoenfeld’s tests. Additionally, we performed tests for effect modification for hypertension, diabetes mellitus, smoking, cholesterol and BMI by including multiplicative interaction terms with these variables and PTH. None of the interaction terms reached statistical significance.

**Sensitivity analysis**

Further, in model D in individuals without chronic kidney disease (1 SD increase of PTH: HR 1.55, 95% CI 1.10-2.19, p = 0.012) or hypercalcemia (HR for 1 SD increase of PTH: 1.52, 95% CI 1.25-1.85, p < 0.0001), respectively, the associations between PTH and vascular disease were similar in all models, as well as when adding CRP, cystatin C, NT-proBNP and troponin I to the model D for the whole cohort (1 SD increase of PTH; HR 1.51, 95% CI 1.24-1.85, p < 0.0001).

The population-attributable risk proportion was calculated as follows: \( p \times \frac{[\text{HR}_{\text{exposed}} - \text{HR}_{\text{unexposed}}]}{\text{HR}_{\text{exposed}}} \), where \( p \) is the proportion of cases that were exposed and \( \text{HR} \) is the hazard ratio adjusted for the established cardiovascular risk factors in multivariable model D.\(^{13}\)

P-values < 0.05 from two-sided tests were considered statistically significant. STATA 12.1 (Stata Corporation, College Station, USA) was used.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
References


