Vitamin D deficiency and hyperparathyroidism are associated with cardiovascular disease (CVD) risk. Low circulating concentrations of 25-hydroxyvitamin D (25(OH)D) and elevated parathyroid hormone (PTH) have been linked to hypertension, insulin resistance, metabolic syndrome, coronary heart disease, congestive heart failure, CVD, and death. Increased vascular stiffness is associated with aging, fragmentation of elastin fibers, and a decrease in the elastin-to-collagen ratio in arterial walls. This process may underlie the development of hypertension, CVD, cerebral dysfunction, and stroke because a rigid arterial tree is less able to accommodate the large pulsatile output from the heart. Increased vascular stiffness accelerates atherogenesis and is associated with an increase in cardiac morbidity and mortality. Vitamin D and PTH are closely linked and may affect vascular smooth muscle tone, endothelial dysfunction, and plaque formation directly linked to hypertension, coronary artery disease, and stroke. Increased vascular stiffness is a plausible mechanism through which 25(OH)D and PTH may affect CVD risk.

Structural and functional alterations in the arterial bed, such as circumferential widening of large arteries and wall thickening, lead to changes in carotid artery distensibility and elasticity, measured with distensibility coefficient (DC) and Young’s elastic modulus (YEM), respectively. These are validated, noninvasive measures of arterial function, which characterize arterial stiffness and can identify individuals at increased CVD risk. Both measure the ability of an artery to expand and contract with each cardiac pulsation; therefore affecting cardiac output, left ventricular stroke work, and blood pressure.

Conclusions—Carotid arterial stiffness is not associated with low 25(OH)D concentrations. Cross-sectional associations between arterial stiffness and high PTH were attenuated by systolic blood pressure. After nearly a decade of follow-up, neither baseline PTH nor 25(OH)D concentrations were associated with progression of carotid arterial stiffness.
Materials and Methods

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

Results

Baseline Characteristics

Baseline characteristics are shown in Table 1. Participants were a mean (SD) of 60.1 (9.4) years old, 54% were female, 39.5% were white, 25.5% were black, 20.5% were Hispanic, and 14.5% were Chinese. The mean annualized 25(OH)D was 26.3 (11.5) ng/mL and was <20 ng/dL in 816 (30%) participants and 20 to 30 ng/mL in 973 (36%) participants. The mean PTH was 43.5 (18.8) pg/dL and was >65 pg/dl in 285 (11%) participants; 86% of subjects graduated from high school and 44% earned <$40000. The average physical activity score was 1665 MET-min/wk. At baseline, the mean DC was 3.1 (1.3)×10⁻³ mm Hg⁻¹, and the mean YEM was 1591 (938) mm Hg.

Cross-Sectional Associations With Arterial Stiffness Measurements

In cross-sectional analyses, continuous 25(OH)D was not associated with stiffness parameters before or after adjustment for CVD risk factors (P>0.1; Tables 2 and 3). When grouped by category of 25(OH)D concentrations, no significant trend toward increasing stiffness with lower 25(OH)D was observed after adjustment for traditional CVD risk factors (P>0.3; Figure). The strongest association with increased stiffness at examination 1 was seen in participants with 25(OH)D concentrations <20 ng/mL (lower DC, β=−1.0×10⁻⁴ mm Hg⁻¹, P=0.01; higher YEM, β=107.2 mm Hg, P=0.03); however, these associations disappeared after adjustment for traditional CVD risk factors (P>0.4). As a continuous variable, 25(OH)D concentration was not associated with arterial stiffness (DC, β=−2.4×10⁻⁷ mm Hg⁻¹, P=0.91; YEM, β=0.2 mm Hg, P=0.92; Tables 2 and 3, cross-sectional model 3).

At baseline, higher PTH concentrations were associated with greater stiffness demonstrated by lower DC (β=−2.5×10⁻⁶ mm Hg⁻¹, P=0.04) and higher YEM (β=1.98 mm Hg, P=0.06; Figure). This relationship seemed to be nonlinear, with overtly elevated PTH concentrations (≥65 pg/mL) being most strongly associated with differences in DC and YEM. Adjusting for CVD risk factors other than blood pressure, PTH >65 pg/mL was associated with lower DC (β=−2.4×10⁻⁴ mm Hg⁻¹, P=0.003) and higher YEM (β=166 mm Hg, P=0.01; Tables 4 and 5). However, these associations no longer were statistically significant when baseline systolic blood pressure (SBP) was included in the model (DC: β=−1.4×10⁻² mm Hg⁻¹, P=0.08; YEM: β=118 mm Hg, P=0.07).

Within race/ethnicity groups, there were no significant associations between baseline 25(OH)D and YEM or DC (all P>0.05). The associations of PTH with DC and YEM seemed strongest for Hispanic participants (DC: β=−3.6×10⁻⁴ mm Hg⁻¹, P=0.02; YEM: β=275 mm Hg, P=0.04), but the P values for the interaction of race/ethnicity with PTH were not statistically significant for DC (P=0.15) or YEM (P=0.08).

Longitudinal Associations With Arterial Stiffness Measurements

DC decreased from 3.1 (1.3)×10⁻³ mm Hg⁻¹ at examination 1 to 2.7 (1.2)×10⁻³ mm Hg⁻¹ at examination 5, and YEM increased from 1591 (938) mm Hg at examination 1 to 1754 (1340) mm Hg at examination 5, both indicating progression of arterial stiffness during the follow-up period. Longitudinal changes in DC and YEM were associated with older age (DC: β=−2.0×10⁻³ mm Hg⁻¹, per year, P<0.0001; YEM: β=13.4 mm Hg, per year, P<0.0001) and higher SBP (DC: β=−2.9×10⁻⁶ mm Hg⁻¹, P=0.007) and use of antihypertensive medication (YEM: β=157.4 mm Hg, P=0.006). No associations or even trends were observed between baseline 25(OH)D or PTH and carotid stiffness (all P>0.3) with or without adjustment for baseline DC and YEM. Additionally, those with baseline PTH >65 pg/mL or 25(OH)D <20 also were not associated with a significant change in DC or change in YEM after nearly 10 years of follow-up (Tables 2 and 3) compared with the reference groups.

Within race/ethnicity groups, no significant associations between 25(OH)D and PTH with longitudinal changes in YEM or DC were observed (all P>0.05), and the P values for the interaction of race/ethnicity with PTH were not statistically significant for changes in DC (P=0.15) or YEM (P=0.96).

Discussion

In the current analysis, we observed a cross-sectional association of higher PTH concentrations with increased arterial stiffness that was independent of CVD risk factors except baseline SBP. No associations were present for 25-OHD. After nearly a decade of aging, neither baseline PTH nor 25-OHD concentrations were associated with changes in arterial stiffening.

Potentially deleterious effects of vitamin D deficiency on CVD risk have been described and have even led some clinicians to promote vitamin D supplementation for CVD risk reduction.15-7,21 A relationship between low vitamin D concentrations and increased arterial stiffness has been described in...
cross-sectional observational studies, however, the effects of vitamin D status on longitudinal changes in arterial stiffness are less clear. Our results are in accordance with small randomized controlled trials of vitamin D supplementation, although the longest of these trials only followed subjects for 3 years. Relationships between vitamin D concentrations and CVD end points have been mixed. For example, low vitamin D concentrations have been associated with increased risk of incident coronary heart disease and presence of coronary artery calcium, but not with congestive heart failure or carotid intima-media.
Table 2. Cross-Sectional and Longitudinal Associations of Serum 25(OH)D Concentrations and Distensibility Coefficient

<table>
<thead>
<tr>
<th>25(OH)D, ng/mL</th>
<th>Cross-Sectional Analyses</th>
<th>Longitudinal Analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Distensibility Coefficient, mm Hg$^{-1}$×10$^{-4}$ (95% Confidence Interval)</td>
<td>Change in Distensibility Coefficient, mm Hg$^{-1}$×10$^{-4}$† (95% Confidence Interval)</td>
</tr>
<tr>
<td>n</td>
<td>Model 1‡</td>
<td>Model 2‡</td>
</tr>
<tr>
<td>≥30.0</td>
<td>918</td>
<td>Ref</td>
</tr>
<tr>
<td>20.0–29.9</td>
<td>973</td>
<td>-0.5</td>
</tr>
<tr>
<td></td>
<td>(−1.5, 0.6)</td>
<td>(−1.0, 1.1)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>816</td>
<td>-1.6#</td>
</tr>
<tr>
<td></td>
<td>(−2.8, −0.4)</td>
<td>(−1.9, 0.5)</td>
</tr>
<tr>
<td>P trend</td>
<td>-</td>
<td>0.01</td>
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</tbody>
</table>

Table 3. Cross-Sectional and Longitudinal Associations of Serum 25(OH)D Concentrations and Young’s Elastic Modulus

<table>
<thead>
<tr>
<th>25(OH)D, ng/mL</th>
<th>Cross-Sectional Analyses</th>
<th>Longitudinal Analyses*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Young’s Elastic Modulus, mm Hg (95% Confidence Interval)</td>
<td>Change in Young’s Elastic Modulus, mm Hg† (95% Confidence Interval)</td>
</tr>
<tr>
<td>n</td>
<td>Model 1‡</td>
<td>Model 2‡</td>
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<td>≥30.0</td>
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<td>20.0–29.9</td>
<td>973</td>
<td>50.2</td>
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<td></td>
<td>(−34.1, 134.6)</td>
<td>(−60.7, 108.7)</td>
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<td>816</td>
<td>107.2#</td>
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<tr>
<td></td>
<td>(11.0, 203.3)</td>
<td>(−38.5, 157.5)</td>
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<tr>
<td>P trend</td>
<td>-</td>
<td>0.03</td>
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</table>

Updated models that include additional covariates...
It may be expected that higher PTH concentrations at baseline would lead to more rapid progression of arterial stiffness during a decade of aging; however, we did not observe a longitudinal association between baseline PTH concentrations and progressive arterial stiffening. Because those with the highest PTH levels also were found to have stiffer arteries at baseline, acceleration of stiffness over time may be blunted because there could be less physiological room for progression of the carotid stiffness parameters (ceiling effect). However, when baseline stiffness parameters were included in the models to attempt to account for this discrepancy, still no associations or even consistent trends were observed. Although PTH and vitamin D were not longitudinally associated with changes in YEM and DC, acceleration of stiffness parameters was observed as expected with traditional CVD risk factors, such as advancing age and hypertension.\textsuperscript{40} Alternatively, although 25(OH)D has a relatively long circulating half-life (≈3 weeks) and is considered a good biomarker, a single measurement may not fully capture cumulative vitamin D exposure during a 10-year period, resulting in misclassification. It is similar for PTH, which comparatively has a much shorter half-life (2–4 minutes), making it even more subject to misclassification. A single measurement seems adequate for cross-sectional associations but is less useful during a decade of follow-up.

Several limitations of the current study should be considered. First, we imaged the carotid arteries but measured brachial artery blood pressure. Brachial artery pressure is considered to be a surrogate for central aortic pressure, but may overestimate stiffness measurements because brachial measurements can overestimate central pressures, although the
Table 4. Cross-Sectional and Longitudinal Associations Between Parathyroid Hormone and Distensibility Coefficient

| PTH, pg/mL | Cross-Sectional Analyses | Longitudinal Analyses* |
|---|---|---|---|
| | (95% Confidence Interval) | (95% Confidence Interval) |
| | n | Beta Parameter | n | Beta Parameter |
| <32.8 | 806 | ref. | ref. | 765 | ref. |
| 32.8–43.7 | 808 | −0.6 | −0.2 | 772 | 1.1# |
| | (−1.7, 0.5) | (−1.3, 0.9) | (−1.3, 0.9) | (0.1, 2.0) |
| 43.8–65.0 | 808 | −1.2# | −0.4 | 777 | 0.4 |
| | (−2.3, −0.0) | (−1.6, 0.7) | (−1.1, 1.1) | (−0.6, 1.4) |
| >65 | 285 | −3.4** | −2.2** | 266 | −0.3 |
| | (−5.0, −1.8) | (−3.8, −0.5) | (−3.0, 0.1) | (−1.6, 1.1) |
| P trend | - | <0.001 | 0.01 | 0.05 | 0.91 |

Model 1 was adjusted for age, sex, race, study field center, education, and income. Model 2 was Model 1 plus physical activity, waist circumference, smoking status, and body mass index. Model 3 was Model 2 plus diabetes mellitus status, antihypertensive medication use, log[c-reactive protein], total cholesterol, high-density lipoprotein–cholesterol, lipid-lowering therapy, and creatinine. Model 4 was Model 3 plus systolic blood pressure.

*Longitudinal analyses shown with adjustment for baseline stiffness measures.
†Between the 2 carotid ultrasounds (9.4 y).
‡Sixty-seven participants missing data on covariates.
§Eighty-three participants missing data on covariates.
¶Seventy-seven participants missing data on covariates.
║Sixty-two participants missing data on covariates.
§Eighty-three participants missing data on covariates.
‡Sixty-seven participants missing data on covariates.
†Between the 2 carotid ultrasounds (9.4 y).

2 measures are highly correlated, especially in older adults. Vitamin D and PTH status were defined by baseline concentrations, and the absence of follow-up levels of either hormone poses a challenge to the interpretation of the longitudinal analyses. This potentially is more likely to be an issue with vitamin D because supplementation is common in the general population, and we did not have information concerning vitamin D supplementation during the follow-up period. Also, the race/ethnicity subgroup analyses may be limited by small sample size. Because all participants had ultrasound studies at

Table 5. Cross-Sectional and Longitudinal Associations Between Parathyroid Hormone and Young’s Elastic Modulus

| PTH, pg/mL | Cross-Sectional Analyses | Longitudinal Analyses* |
|---|---|---|---|
| | (95% Confidence Interval) | (95% Confidence Interval) |
| | n | Beta Parameter | n | Beta Parameter |
| <32.8 | 806 | ref. | ref. | 765 | ref. |
| 32.8–43.7 | 808 | 39.2 | 20.1 | 772 | −63.5 |
| | (−50.9, 129.4) | (−70.0, 110.1) | (−72.3, 108.1) | (−184.3, 57.2) |
| 43.8–65.0 | 808 | 63.4 | 28.4 | 777 | −59.0 |
| | (−28.7, 155.5) | (−64.2, 121.5) | (−62.2, 97.5) | (−182.1, 64.2) |
| >65 | 285 | 217.0# | 159.0** | 266 | −63.9 |
| | (88.1, 345.9) | (28.8, 289.2) | (35.6, 296.7) | (−88.3, 264.6) |
| P trend | - | 0.002 | 0.03 | 0.55 | 0.64 |

Model 1 was adjusted for age, sex, race, study field center, education, and income. Model 2 was Model 1 plus physical activity, waist circumference, smoking status, and body mass index. Model 3 was Model 2 plus diabetes mellitus status, antihypertensive medication use, log[c-reactive protein], total cholesterol, high-density lipoprotein–cholesterol, lipid-lowering therapy, and creatinine. Model 4 was Model 3 plus systolic blood pressure.

*Longitudinal analyses shown with adjustment for baseline stiffness measures.
†Between the 2 carotid ultrasounds (9.4 y).
‡Sixty-seven participants missing data on covariates.
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‡Sixty-seven participants missing data on covariates.
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exams 1 and 5, there may be a survivorship bias. Participants who were followed up to examination 5 were healthier and less likely to have a nonfatal CVD event than the complete MESA cohort, which would likely result in a null bias.

Conclusions

In cross-sectional analyses, we did not observe any independent associations between arterial stiffness measures and vitamin D status. Carotid arterial stiffness was associated with PTH concentrations >65 mg/dL; however, the associations were attenuated by adjustment for SBP. In longitudinal analyses, advancing age and hypertension were associated with progression of arterial stiffness; however, neither baseline PTH nor 25(OH)D was associated with changes in arterial stiffness measures after nearly a decade of follow-up. Elevated PTH is associated with carotid stiffness; however, the causal and temporal inter-relationships of PTH, blood pressure, and carotid stiffness are not entirely clear and warrant further study.

Acknowledgments

We thank the other investigators, the staff, and the participants of the Multi-Ethnic Study of Atherosclerosis (MESA) study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

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Disclosures

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References

Low vitamin D and high parathyroid hormone concentrations have been associated with heart disease and hypertension, but much less is known about their long-term effects on arterial stiffening, which has been linked to the development of heart failure, strokes, and heart attacks. In this study, carotid artery stiffness was associated with high parathyroid hormone levels, although this finding was attenuated by systolic blood pressure. Vitamin D concentration was not associated with baseline arterial stiffness. Neither baseline parathyroid hormone nor vitamin D concentrations were associated with changes in arterial stiffening during nearly a decade of follow-up. These findings suggest that parathyroid hormone may impact the development of arterial stiffness; however, the causal and temporal inter-relationships of parathyroid hormone, blood pressure, and carotid stiffness are not entirely clear and warrant further study.

Significance
Adam D. Gepner, Laura A. Colangelo, Marc Blondon, Claudia E. Korcarz, Ian H. de Boer, Bryan Kestenbaum, David S. Siscovick, Joel D. Kaufman, Kiang Liu and James H. Stein

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Materials and Methods

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

Study participants and design

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal cohort study that is investigating the prevalence, correlates, and progression of subclinical cardiovascular disease in a population-based sample of 6,814 men and women aged 45 to 84 years who were free of known cardiovascular disease at its inception in 1999. MESA recruited participants from 6 United States communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; Northern Manhattan, New York; and St. Paul, Minnesota). The study objectives and design have been published previously.1 All participants gave informed consent for the study protocol, which was approved by the institutional review boards of the University of Wisconsin ultrasound reading center and all MESA field centers. This analysis includes the subset of MESA participants with baseline serum 25(OH)D (n=6,473)2 and valid carotid artery distensibility measurements at exams 1 (n=2,707) and 5 (n=2,580) which occurred, on average, 9.4 (0.5) years apart.3,4

Measurement of 25-Hydroxyvitamin D and Parathyroid Hormone Concentrations

Detailed methods for collection and measurement of concentrations of serum 25(OH)D has been previously described.2 Briefly, 25(OH)D concentrations were measured by mass spectrometry from frozen serum collected at Exam 1, calibrated to NIST standards (interassay CV <3.4%).5 Serum PTH was measured by two-site immunoassay on a Beckman DxC automated platform (inter-assay CV 6.1% at 30.1 pg/ml and 3.4% at 94.5 pg/ml).6 As season-specific thresholds for 25-OHD may be most relevant,7 25-OHD was adjusted for seasonal variation using an algorithm derived and internally validated in MESA.2

B-mode Ultrasound and Brachial Blood Pressure Measurements

A detailed protocol has been published previously.3 At exam 1, B-mode ultrasound video loop recordings of a longitudinal section of the distal right common carotid artery were recorded on videotape using a Logiq 700 ultrasound system (General Electric Medical Systems, transducer frequency 13 MHz). Video images were digitized at high resolution and frame rates using a Medical Digital Recording (MDR) device (PACSGEAR, Pleasanton, CA) and converted into DICOM compatible digital records. At exam 5, a similar protocol was performed using the same ultrasound and digitizing equipment; however, the video output was directly digitized using the same MDR settings without use of videotape. Centrally trained and certified sonographers from all 6 MESA sites used selected reference images from exam 1 to try to match the scanning conditions of the initial study, including common carotid artery display depth, angle of approach, surrounding tissues and internal landmarks, degree of jugular venous distension, and ultrasound system settings. After 10 minutes of rest in the supine position and immediately before the ultrasound image acquisition, repeated measures of brachial blood pressures were obtained using a standardized protocol with an automated upper arm sphygmomanometer (DINAMAP, GE Medical Systems, Milwaukee, WI). Ultrasound images were reviewed and interpreted by the MESA Carotid Ultrasound Reading Center (the University of Wisconsin Atherosclerosis Imaging Research Program, Madison, WI). Systolic and diastolic diameters were determined as the largest and smallest diameters during the cardiac cycle. All measurements were performed in triplicate from 2-3 consecutive cardiac cycles to derive mean internal diameter at peak systole and mean internal and external diameters at end-diastole using Access Point Web version 3.0 (Freeland Systems, LLC).
**Measurement of Carotid Distensibility and Young’s Elastic Modulus**

The carotid distensibility coefficient (DC) was calculated as:

\[
DC = \frac{(Ds^2 - Dd^2)}{\Delta p} \cdot \frac{Ds}{Dd^2}
\]

Where \(Ds\) represents the internal arterial diameter at peak systole, \(Dd\) represents the internal diameter at end-diastole, and \(\Delta p\) represents the difference between the brachial artery SBP and diastolic blood pressure measurements (pulse pressure).\(^8\)

Young’s elastic Modulus (YEM), the ratio of stress and circumferential strain in the arterial wall, was calculated as:

\[
YEM = \frac{h}{Dd}
\]

Where \(Dd\) is the internal arterial diameter at end-diastole, \(h\) is the arterial wall thickness at end-diastole (external carotid artery diameter minus internal carotid artery diameter).\(^8,9\)

YEM and DC are inversely related; thus, increased arterial stiffness corresponds to a lower DC and a higher YEM.

Reproducibility measurements were performed by a single reader with 25 blinded, replicate images. Reproducibility was excellent for internal end-diastolic diameter (mean [SD]: 0.589 [0.070] cm, \(r=0.998, p<0.0001\)), peak systolic internal diameter (0.637 [0.074] cm, \(r=0.998, p<0.0001\)), end-diastolic external diameter (0.743 [0.081] cm, \(r=0.997, p<0.0001\)), change in diameter (0.048 [0.016] cm, \(r=0.925, p<0.0001\)) and wall thickness (0.154 [0.033] cm, \(r=0.989, p<0.0001\)). Only rare outliers were seen on inspection of Bland-Altman plots.

**Covariates**

Demographic, medical history, and laboratory data were obtained from the first (July, 2000 to August, 2002) and fifth (January, 2012 to February, 2012) examinations of the MESA cohort. Prescription medications including antihypertensive, diabetic, and lipid-lowering agents were verified. A diagnosis of hypertension was defined as SBP \(\geq 140\) mmHg, diastolic blood pressure \(\geq 90\) mmHg, or the use of antihypertensive medications. Diabetes mellitus was defined as fasting blood glucose \(\geq 126\) mg/dL or the use of antiglycemic medications. Impaired fasting glucose was defined as blood glucose from \(\geq 100\) but <126 mg/dL. Total and high-density lipoprotein cholesterol (HDL-C) levels were measured from blood samples obtained after a 12-hour fast. Low-density lipoprotein cholesterol was calculated using the Friedewald equation.\(^10\)

**Statistical Analysis**

Results are reported as mean (standard deviation) for continuous variables or percentages for categorical variables. Serum 25(OH)D and PTH were assessed as a continuous and categorical variable with commonly used cut points (25(OH)D: < 20 ng/mL, 20-30 ng/mL, and \(\geq 30\) ng/mL; PTH: 65pg/ml and tertiles).\(^6,11,12\) Categorical cut points were predefined, based on clinically relevant thresholds, as well as prior MESA publications.

Cross-sectional analysis of associations between 25(OH)D and the outcome variables, carotid distensibility and YEM were assessed at Exam 1 using linear regression models. Differences between baseline and exam 5 measures were examined using analysis of covariance (ANCOVA), adjusted for risk factors, with and without adjustment for baseline stiffness measures. All models were pre-specified and sequentially performed as 1) adjusted for sex, race, study field center, education, and income; 2) physical activity, waist circumference, smoking status, and BMI; 3) diabetes mellitus status, antihypertensive medication use, log[C-reactive protein], total cholesterol, HDL-C, lipid lowering therapy, and creatinine; and 4) adding in baseline systolic blood pressure. Tests of trend over the 25(OH)D and PTH categories were done by assigning the median value to each individual in a given category and modeling this as a continuous variable. Statistical significance was set at \(P<0.05\). All analyses were carried out with the use of SAS (Version 9.3, Cary, NC: SAS Institute Inc.).
References


