Association of serum vitamin D with diagnosis and growth of abdominal aortic aneurysm

Shivshankar Thanigaimani, PhD,[a,b] Rachel E. Neale, PhD,[c,d] Mary Waterhouse, PhD,[c] Joseph V. Moxon, PhD,[a,b] Bu B. Yeap, PhD,[e,f] Paul E. Norman, PhD,[e] Leon Flicker, PhD,[e] Graeme J. Hankey, PhD,[e,g] Jason Jenkins, PhD,[h] Frank Quigley, PhD,[i] Michael W. Clarke, PhD,[j,k] *and* Jonathan Golledge, MA, FRCS, FRACS,[a,b,l] *Townsville and Brisbane, QLD, and Perth, WA, Australia*

ABSTRACT

Objective: We examined the associations between 25-hydroxy vitamin D ($25(OH)D_3$) concentration and the diagnosis and growth of abdominal aortic aneurysm (AAA).

Methods: AAA cases and healthy controls were recruited from vascular centers or the community. A subset of participants with AAA were monitored by repeat ultrasound examination to assess AAA growth. Serum $25(OH)D_3$ concentration was measured using a validated mass spectrometry method and categorized into guideline-recommended cut-points after deseasonalization. The associations between deseasonalized $25(OH)D_3$ concentration and AAA diagnosis and growth were examined using logistic regression and linear mixed effects modeling.

Results: A total of 4673 participants consisting of 873 (455 controls and 418 cases) from Queensland and 3800 (3588 controls and 212 cases) from Western Australia were recruited. For every 1 standard deviation increase in $25(OH)D_3$ concentration, odds of AAA diagnosis was significantly reduced in both Queensland (adjusted odds ratio: 0.81; 95% confidence interval [CI]: 0.69-0.95; $P = .009$) and Western Australia (adjusted odds ratio: 0.80; 95% CI: 0.68-0.94; $P = .005$) cohorts. A subset of 310 eligible participants with small AAA from both regions were followed for a median of 4.2 (interquartile range: 2.0-5.8) years. Compared with vitamin D sufficient participants (50 to <75 nmol/L), annual mean AAA growth was significantly greater in those with higher vitamin D (≥75 nmol/L) (adjusted mean difference: 0.1 mm/y, 95% CI: 0.1-0.2; $P < .001$).

Conclusions: High $25(OH)D_3$ concentration was paradoxically associated with a lower likelihood of AAA diagnosis and faster AAA growth. Further research is needed to resolve these conflicting findings. (JVS—Vascular Science 2024;5:100208.)

Clinical Relevance: The findings of this study suggest that relative vitamin D deficiency increases the risk of abdominal aortic aneurysm diagnosis, but paradoxically high circulating markers of vitamin D are associated with faster aneurysm growth. These findings support the need for vitamin D sufficiency not excess, but need validation in other cohorts before incorporation into clinical management protocols.

Keywords: Vitamin D; Abdominal aortic aneurysm; AAA diagnosis; AAA growth

The prevalence of abdominal aortic aneurysm (AAA) is estimated to be approximately 1.4% in people aged between 50 and 84 years.[1] In 2010, the estimated global death rate related to aortic aneurysm was 2.78 per 100,000 individuals, and the risk was increased to 17.2 per 100,000 in men aged between 65 and 69 years.[2]

From the Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry,[a] and Australian Institute of Tropical Health and Medicine,[b] James Cook University, Townsville; the Population Health Department, QIMR Berghofer Medical Research Institute,[c] and the School of Public Health, University of Queensland,[d] Brisbane; the Medical School, University of Western Australia,[e] the Department of Endocrinology and Diabetes, Fiona Stanley Hospital,[f] and the Perron Institute for Neurological and Translational Science,[g] Perth; the Department of Vascular Surgery, Royal Brisbane and Women's Hospital, Brisbane[h]; the Mater Hospital, Townsville[i]; the Metabolomics Australia, Centre for Microscopy, Characterisation and Analysis,[j] and School of Biomedical Sciences, Faculty of Health and Medical Sciences,[k] The University of Western Australia, Perth; and the Department of Vascular and Endovascular Surgery, Townsville University Hospital, Townsville.[l]

J.G. is supported by grants from the National Health and Medical Research Council (NHMRC), National Heart Foundation, Queensland Government, Medical Research Futures Fund, and Townsville Hospital and Health Services. The D-Health Trial is funded by NHMRC project grants (GNT1046681, GNT1120682). The Health in Men Study was supported by project grants from the NHMRC (1128083, 1045710). M.W.C. is affiliated to Metabolomics Australia, University of Western Australia, Perth, Western Australia, Australia, which was supported by infrastructure funding from the Western Australian State Government in partnership with the Australian Federal Government, through Bioplatforms Australia and the National Collaborative Research Infrastructure Strategy (NCRIS). Funding sources were not involved in the design, collection, analysis, or interpretation of data included in the manuscript. The authors are solely responsible for this paper and its final contents.

Additional material for this article may be found online at www.jvsvs.org.

Correspondence: Jonathan Golledge, MA, FRCS, FRACS, Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, 1 James Cook Dr, Douglas, QLD 4814, Australia (e-mail: jonathan.golledge@jcu.edu.au).

The editors and reviewers of this article have no relevant financial relationships to disclose per the JVS-Vascular Science policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2666-3503

https://doi.org/10.1016/j.jvssci.2024.100208

The only current treatment for AAA is open or endovascular surgery, with randomized controlled trials reporting no convincing evidence that any drug therapy limits AAA growth or rupture.[3] Current clinical guidelines recommend repeat monitoring of small asymptomatic AAA (<55 mm in men and <50 mm in women) and consideration of surgical repair for symptomatic, ruptured, or large asymptomatic AAA.[4] Identifying modifiable risk factors for AAA may lead to new methods to prevent AAA and limit aneurysm growth.

A number of animal studies have suggested that low vitamin D status may be involved in the pathogenesis of AAA.[5,6] In one mouse-model study, animals that were fed with a diet that led to vitamin D deficiency had substantially larger and more rupture-prone AAAs compared with control mice.[5] Activation of the vitamin D receptor has been reported to reduce the development of angiotensin II-induced AAA and was associated with reduced aortic inflammation and extracellular matrix remodeling.[6] In a previous observational study in humans, we reported an association between low 25-hydroxy vitamin D (25(OH)D_3) concentration and large AAA,[7] a finding confirmed in a pooled analysis of four case-control studies.[8] In contrast, the Atherosclerosis Risk in Communities study reported that markers of vitamin D metabolism were not associated with AAA diagnosis.[9] These studies differed in population, recruitment method, and data collection, which may explain the disparate findings. Given these prior contradictory results, further studies are needed to examine any role of vitamin D deficiency in AAA pathogenesis.[7-9] Furthermore, no study has examined the association between 25(OH)D_3 concentration and AAA growth. This is important because an unmet clinical need is a lack of knowledge about the role of lifestyle modification or interventions to limit the progression of established AAAs. The aims of this study were to examine the associations between 25(OH)D_3 concentration and AAA diagnosis and growth.

MATERIALS AND METHODS

Study design and participant recruitment. The flow diagram illustrating the patient population recruited for this study is presented in Fig. To assess the association between 25(OH)D_3 concentration and risk of AAA (aim 1), we conducted a case-control study in participants from Queensland and Western Australia (WA). Participants with an aortic diameter of ≥30 mm confirmed by ultrasound imaging were considered to have an AAA.

In Queensland, AAA cases were recruited from an ongoing prospective cohort study that aims to identify novel risk factors for peripheral vascular disease diagnosis and outcomes.[10] Control participants were selected from the D-Health Trial cohort. The D-Health Trial recruited Australians aged 60 years or more, primarily from a population register, although a small number of volunteers were also recruited (ACTRN12613000743763).[11,12] We selected controls who were residents of Queensland and who had provided a blood sample. The aortic diameter of Queensland controls was not assessed.

In WA, AAA cases were recruited from the Health in Men Study (HIMS), which was formed from a population screening trial for AAA in WA.[13] Controls were also identified from the HIMS as having aortic diameter <30 mm on ultrasound imaging.[4]

To analyze the association between 25(OH)D_3 and AAA growth (aim 2), we included a subset of AAA cases (aortic diameter ≥30 mm) from the case-control study who underwent at least two ultrasound scans a minimum of 6 months apart. The study was conducted with approval from the ethics committees of Townsville Hospital and Health Services, James Cook University, University of Western Australia, and the QIMR Berghofer Medical Research Institute. AAA cases were recruited from outpatient clinics at Townsville University Hospital, Royal Brisbane and Women's Hospital, and the Mater Hospital Townsville between February 2008 and November 2015 and the HIMS between 1996 and 1999. The study was performed in accordance with the Declaration of Helsinki, and all participants provided informed consent, either written or electronically.

AAA imaging protocol. Experienced sonographers, who were unaware of the participants' 25(OH)D_3 concentrations, measured maximum infrarenal aortic diameters in the anterior to posterior and transverse orthogonal planes as previously described.[14] The aortic diameter was measured from the outer to outer walls of the infrarenal aorta as previously described.[15] Excellent reproducibility of these measurements has been reported.[15] AAA was diagnosed by the maximum infrarenal aortic diameter ≥30 mm.

Serum 25-hydroxy vitamin D assessment. Serum concentration of 25(OH)D_3 was measured at Metabolomics (WA, Australia) using a validated assay.[16] The assay was performed using two-dimensional ultraperformance liquid chromatography separation coupled tandem mass spectrometry as previously described.[16] The lower limit of quantification for 25(OH)D_3 was 2.0 nmol/L. For deseasonalization, we applied a previously validated method in which a sinusoidal model was fitted using the formula: Actual 25(OH)D_3 concentration = $\beta_0 + \beta_1 \sin\left(\frac{2\pi t}{12}\right) + \beta_2 \cos\left(\frac{2\pi t}{12}\right)$, where t was the month of blood collection. We then added the overall mean of 25(OH)D_3 concentrations to the obtained residuals from the sinusoidal model.[17] Month-specific mean values of raw and deseasonalized 25(OH)D_3 concentrations of the included cases and controls are provided in Supplementary Table I (online only). For analysis of both aims 1 and 2, we treated deseasonalized 25(OH)D_3

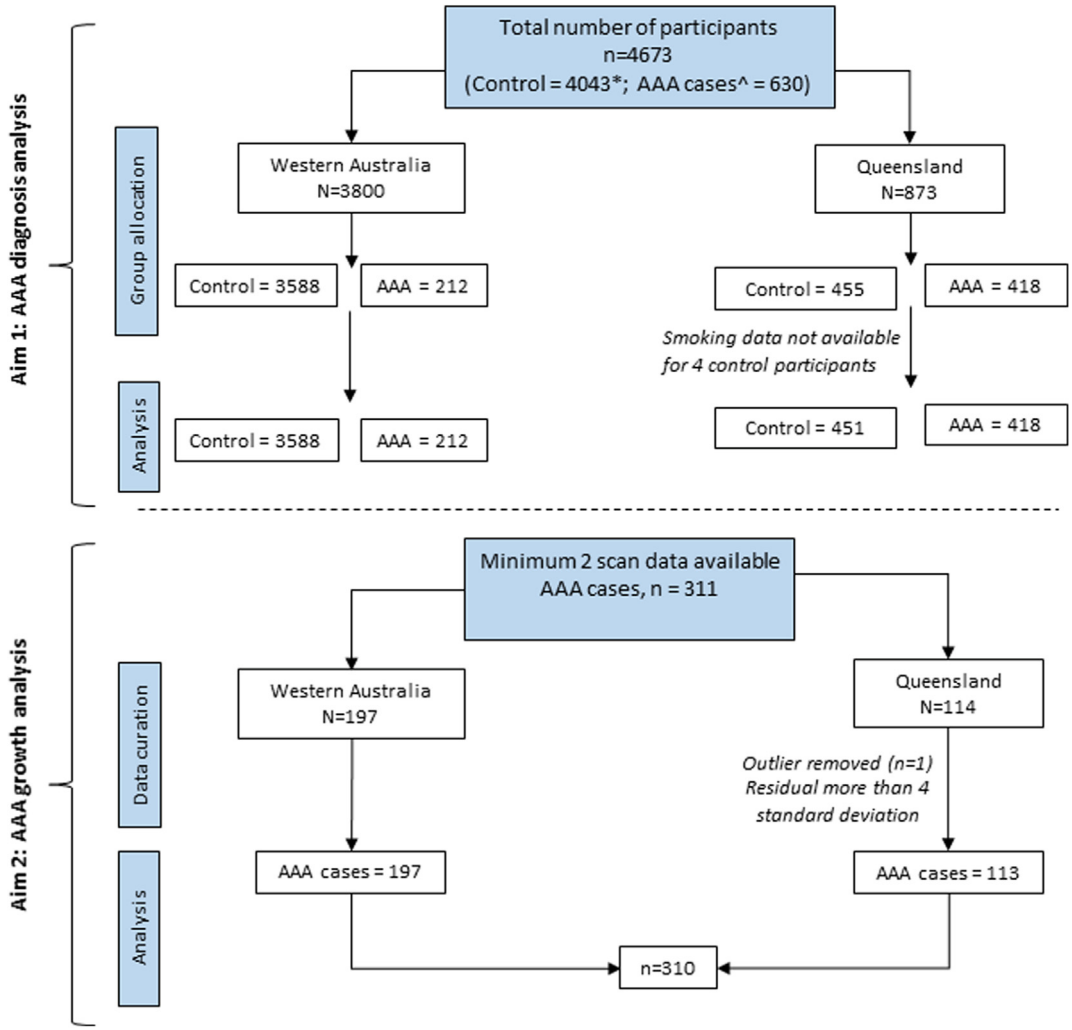


Fig. Flow diagram illustrating the allocation of the study participants from Queensland and Western Australia to the analyses assessing the association between 25(OH)D_3 concentrations and abdominal aortic aneurysm (AAA) diagnosis and growth. *Aortic diameter not available for Queensland controls. ^AAA confirmed by ultrasound imaging. *25(OH)D_3*, 25-Hydroxy vitamin D.

concentration as a continuous and a categorical variable. For categorization, we used the established guideline-recommended cut-points as follows: high ≥75 nmol/L, sufficient 50 to <75 nmol/L, and low <50 nmol/L.

Ascertainment of covariates. For the main analysis, covariates including age, sex, history of smoking, diabetes, and hypertension were collected. Additionally for AAA cases, history of ischemic heart disease, stroke, and prescription of aspirin, other antiplatelet medications, β-blockers, statins, and fibrates were also collected from their medical history after clinical interview and physical examination. In the WA cohort only, number of years smoked, number of cigarettes smoked per day, systolic blood pressure, and prior diagnosis of osteoporosis or Paget's disease were collected. Number of years smoked and number of cigarettes smoked per day were used to estimate pack-years using the following formula: Number of cigarettes per day/20 * Number of years smoked.[18] Participants' smoking history was divided into nonsmokers, <20, 21-70, and >70 pack-years.

Statistical analyses. We compared the characteristics of AAA cases and controls using the Mann-Whitney *U* or Kruskal-Wallis test (continuous variables) and the χ^2 or Fisher exact test (categorical variables). Associations between deseasonalized 25(OH)D_3 concentration and outcomes were analyzed using logistic regression (AAA diagnosis) and linear mixed effects (LME) modeling (AAA growth). In LME models, time was considered a fixed effect, and participants were treated as random effects. For both logistic and linear regression models, the outcome measures were estimated for every 1 standard deviation (SD, calculated region-wise for diagnosis, and combined for growth analysis) increase in deseasonalized 25(OH)D_3 concentration and according to 25(OH)D_3

Table I. Characteristics of all participants recruited for determining the association between deseasonalized 25(OH)D_3 concentration and abdominal aortic aneurysm (AAA) diagnosis

Characteristic	Overall			Queensland participants			Western Australia participants		
	Controls (n = 4043)	Cases (n = 630)	*P*	Controls (n = 455)	Cases (n = 418)	*P*	Controls (n = 3588)	Cases (n = 212)	*P*
Age, years	70.0 (67.0-74.0)	73.0 (68.0-77.0)	<.001	72.0 (68.0-77.0)	74.0 (68.0-78.9)	.039	70.0 (67.0-73.0)	71.5 (68.0-74.0)	<.001
Male sex, n (%)	3809 (94.2)	569 (90.3)	<.001	221 (48.6)	357 (85.4)	<.001	3588 (100.0)	212 (100.0)	–
Smoking, n (%)			<.001			<.001			<.001
Never	1567 (38.8)	84 (13.3)		247 (54.3)	55 (13.2)		1320 (36.8)	29 (13.7)	
Past	2206 (54.6)	284 (45.1)		195 (42.9)	253 (60.5)		2011 (56.0)	31 (14.6)	
Current	266 (6.6)	262 (41.6)		9 (2.0)	110 (26.3)		257 (7.2)	152 (71.7)	
Hypertension, n (%)	1506 (37.2)	394 (62.5)	<.001	220 (48.3)	290 (69.4)	<.001	1286 (35.8)	104 (49.1)	<.001
Diabetes, n (%)	335 (8.3)	102 (16.4)	<.001	42 (9.2)	78 (18.7)	<.001	293 (8.2)	24 (11.3)	.137
Serum 25(OH)D_3, nmol/L			.001			.071			<.001
Median (Q1-Q3)	68.8 (54.5-84.1)	72.3 (57.3-89.1)		81.1 (64.7-95.8)	78.6 (63.4-93.0)		67.6 (53.1-82.0)	59.6 (46.3-74.9)	
Low (<50)	719 (17.8)	98 (15.5)		28 (6.2)	40 (10.1)		691 (19.3)	58 (27.4)	
Sufficient (50 to <75)	1790 (44.3)	241 (38.3)		157 (34.5)	147 (34.2)		1633 (45.5)	94 (44.3)	
High (≥75)	1534 (37.9)	291 (46.2)		270 (59.3)	231 (55.7)		1264 (35.2)	60 (28.3)	

25(OH)D_3, 25-Hydroxy vitamin D; *Q1*, first quartile; *Q3*, third quartile.
Continuous variables are shown as median (first quartile – third quartile). Nominal variables are shown as count and percentage. *P* values represent overall comparisons between the groups using the Mann-Whitney *U* or Kruskal-Wallis test (continuous variables) and the χ^2 or Fisher exact test (categorical variables). History of hypertension and diabetes were based on self-reporting from participants. Those who did not report a history were classified as not having the comorbidity. Smoking data were missing for four control participants.

category. For aim 1, age, sex, history of smoking, hypertension, and diabetes were included in the multivariable model, and the results are presented as odds ratios and 95% confidence intervals (CIs) for each region separately. Results from the two states were meta-analyzed using a random effects model, and outcomes were reported as odds ratios and 95% CIs. For aim 2, key clinical risk factors including age, sex, history of smoking, diabetes, and initial AAA diameter were included in the multivariable model, and the data from both regions were analyzed together to improve the power. Differences in AAA growth are reported as estimates of mean difference (MD) in annual growth (95% CI). Outliers higher than 4 SD of residuals from the adjusted model were removed from the analysis. We used qq-norm plots to assess the normality of residuals from the LME models. Because of the marked difference between men and women in the risk of AAA and the small number of women with growth data available, we performed additional analyses restricted to men. Hartigan's dip test was performed to assess if the data set had more than one mode in its distribution due to sampling from two different states.[19] The dip statistic (D) essentially measures the distance between the empirical cumulative density function (probability distribution of random variables) and the closest unimodal cumulative density function where significance represents the presence of more than one mode. Sensitivity analyses were performed to adjust for systolic blood pressure and pack-years smoked. Participants with a prior diagnosis of osteoporosis or Paget's disease were excluded from the sensitivity analyses. Statistical analyses were conducted using R version 4.1.3 (R Foundation for Statistical Computing) employing the "nlme" and "ggplot2" packages.[20,21] Statistical significance was considered to be $P \leq .05$.

RESULTS

Study participants

A total of 4673 participants of whom 630 had an AAA and 4043 were controls were recruited. Compared with controls, AAA cases were significantly more likely to have a history of smoking, hypertension, and diabetes. The median age of AAA cases was significantly higher than that of controls (Table I). Similar differences between cases and controls were observed in both Queensland and WA cohorts (Table I).

For aim 2, a total of 311 AAA cases who had undergone monitoring of their AAA with at least two ultrasound scans were identified. After removing the outliers, 310 participants were eligible for analysis. This cohort was followed for a median of 4.2 (interquartile range: 2.0, 5.8) years, during which they had a median of 5 (interquartile range: 3, 7) scans. Characteristics of participants in this cohort, overall and according to tertiles of serum 25(OH)D_3 concentration, are provided in Table II. The aortic diameter of AAA cases was significantly higher in Queensland compared with WA cases (48.6 ± 12.9 vs 37.3 ± 8.6; $P < .001$).

Association between 25(OH)D_3 concentration and AAA diagnosis

Queensland cohort. A total of 873 participants (455 controls and 418 AAA cases) were recruited from Queensland, but 4 controls were missing smoking data that was

Table II. Characteristics of the subset of participants (n = 311) included in the analysis of the association between deseasonalized 25(OH)D_3 concentration and annual mean abdominal aortic aneurysm (AAA) growth (aim 2)

Risk factors	N (%)	25 (OH)D_3 tertile (nmol/L)			*P*
		Tertile 1, <55.8 (n = 73)	Tertile 2, 55.8-74.1 (n = 122)	Tertile 3, >74.1 (n = 116)	
Age, years, median (Q1-Q3)	73.0 (69.0-77.0)	75.0 (69.3-79.3)	75.2 (68.3-79.2)	75.7 (71.4-80.0)	.404
Baseline AAA diameter, mm, median (Q1-Q3)	36.5 (31.3-42.1)	40.8 (37.9-42.3)	42.1 (39.8-46.8)	44.6 (40.1-48.1)	.317
Male sex	295 (94.9)	71 (97.3)	119 (97.5)	105 (90.5)	.028
Smoking					.332
Never	45 (14.5)	15 (20.8)	15 (12.3)	15 (12.9)	
Past	216 (69.4)	49 (68.0)	89 (73.0)	78 (67.3)	
Current	50 (16.1)	9 (12.5)	18 (14.7)	23 (19.8)	
Hypertension	155 (49.8)	42 (57.5)	63 (51.6)	50 (43.1)	.136
Diabetes	45 (14.5)	6 (8.2)	18 (14.8)	21 (18.1)	.170
Ischemic heart disease	124 (39.9)	32 (43.8)	51 (41.8)	41 (35.3)	.436
Aspirin	149 (47.9)	31 (42.5)	65 (53.3)	53 (45.7)	.286
Other antiplatelet medications	41 (13.2)	8 (11.0)	13 (10.7)	20 (17.2)	.264
β-Blockers	80 (25.7)	15 (20.5)	33 (27.0)	32 (27.6)	.510
Statins	183 (58.8)	39 (53.4)	80 (65.6)	64 (55.2)	.149
Fibrates	5 (1.6)	1 (1.4)	2 (1.6)	2 (1.7)	.982
Serum 25(OH)D_3 concentration, nmol/L, median (Q1-Q3)	68.0 (51.4-84.2)	54.7 (47.8-63.7)	79.4 (74.8-85.7)	106.0 (99.2-116.5)	<.001
Deficient <50	73 (23.5)	73 (100.0)	25 (20.5)	0 (0.0)	
Sufficient 50 to <75	122 (39.2)	0 (0.0)	94 (77.0)	0 (0.0)	
High ≥75	116 (37.3)	0 (0.0)	3 (2.5)	116 (100.0)	

25(OH)D_3, 25-Hydroxy vitamin D; *Q1*, first quartile; *Q3*, third quartile.
Continuous variables are shown as median (first quartile – third quartile). Nominal variables are shown as count and percentage. The column N (%) does not apply for age and baseline AAA diameter. There were no missing data in the presented variables. The Kruskal-Wallis test (continuous variables) and the χ^2 test (categorical variables) were used for calculating *P* values.

included as a covariate, so the analysis was restricted to 869 participants. The median (first quartile – third quartile) deseasonalized 25(OH)D_3 concentration was not significantly different in the controls than in the AAA cases (81.1 [64.7-95.8] nmol/L vs 78.6 [63.4-93.0] nmol/L; Mann-Whitney *U*, $P = .071$). For every 1 SD (25.3 nmol/L) increase in deseasonalized 25(OH)D_3 concentration, the odds of having an AAA was significantly reduced in both unadjusted and unadjusted models (Table III). Compared with those with sufficient 25(OH)D_3 concentration, the odds of having an AAA was significantly greater in participants with low 25(OH)D_3 and nonsignificantly lower in those with high 25(OH)D_3 concentrations. The findings were similar in analyses restricted to men, although with wider CIs (Table III).

WA cohort. A total of 3800 male participants (3588 controls and 212 AAA cases) were recruited from WA. The median (first quartile – third quartile) deseasonalized 25(OH)D_3 concentration was significantly higher in the controls than in the AAA cases (67.6 [53.1-82.0] nmol/L vs 59.6 [46.3-74.9] nmol/L; Mann-Whitney *U*, $P < .001$). For every 1 SD (22.6 nmol/L) increase in deseasonalized 25(OH)D_3 concentration, the odds of having an AAA was significantly reduced in both unadjusted and adjusted models (Table IV). In analyses where 25(OH)D_3 concentration was categorized, we observed that compared with those with sufficient concentration, the odds of having an AAA was higher in participants with low concentrations, and odds were lower in those with high 25(OH)D_3 concentration (Table IV), although the CIs were consistent with a null finding.

Association between 25(OH)D_3 concentration and AAA growth in Queensland and WA

We observed a significant positive association between deseasonalized 25(OH)D_3 concentration and annual mean AAA growth (risk factor-adjusted MD for the increase in every 1 SD of 25(OH)D_3 [23.84 nmol/L]: 0.1 mm/y, 95% CI: 0.0, 0.1; $P < .001$) (Table V). Compared with those who were vitamin D sufficient, annual mean AAA growth was not significantly different in those who had low vitamin D. In contrast, having high vitamin D concentrations was associated with significantly faster AAA growth (adjusted MD: 0.1 mm/y, 95% CI: 0.1, 0.2; $P < .001$). The findings for men (n = 295) were similar (Table V).

Table III. Association between continuous and categorical deseasonalized 25(OH)D_3 concentration and abdominal aortic aneurysm (AAA) diagnosis in participants from Queensland

	Unadjusted model			Adjusted model		
25 (OH)D_3	OR	95% CI	*P*	OR	95% CI	*P*
All Queensland participants (451 controls vs 418 cases)						
Per 1 SD increase	0.87	0.76-0.99	.043	0.81	0.69-0.95	.009
<50 nmol/L	1.53	0.90-2.62	.120	1.89	1.03-3.54	.043
50 to <75 nmol/L			Reference			
≥75 nmol/L	0.91	0.69-1.22	.536	0.91	0.65-1.27	.578
Men only (219 controls vs 357 cases)						
<50 nmol/L	1.34	0.68-2.75	.412	1.70	0.80-3.74	.176
50 to <75 nmol/L			Reference			
≥75 nmol/L	0.98	0.68-1.40	.899	0.90	0.61-1.32	.584

25(OH)D_3, 25-Hydroxy vitamin D; *CI*, confidence interval; *OR*, odds ratio; *SD*, standard deviation.
Adjusted model included age, sex, history of smoking, hypertension, and diabetes. Four participants were excluded from analysis due to missing smoking data.

Table IV. Association between continuous and categorical deseasonalized 25(OH)D_3 concentration and abdominal aortic aneurysm (AAA) diagnosis in male participants from Western Australia

	Unadjusted model			Adjusted model		
25 (OH)D_3	OR	95% CI	*P*	OR	95% CI	*P*
All Western Australia participants (3588 controls vs 212 cases)						
Per 1 SD increase	0.76	0.65-0.88	.001	0.80	0.68-0.94	.005
<50 nmol/L	1.46	1.03-2.04	.029	1.30	0.92-1.84	.133
50 to <75 nmol/L			Reference			
≥75 nmol/L	0.82	0.59-1.15	.255	0.84	0.60-1.17	.301

25 (OH)D_3, 25-Hydroxy vitamin D; *CI*, confidence interval; *OR*, odds ratio; *SD*, standard deviation.
Adjusted model included age, history of smoking, hypertension, and diabetes.

The model fitness, assessed using a qq plot, showed significant skewness toward both tail ends, suggesting that the sample data came from a mixture of two different distributions (Supplementary Fig, online only). Hartigans' dip test for unimodality showed $P = .004$, suggesting that the residual distribution of the included data set was significantly likely to have at least more than one mode.

Sensitivity analyses

Sensitivity analyses suggested that a 1 SD higher 25(OH)D_3 concentration was associated with a lower likelihood of AAA diagnosis in the adjusted model after excluding participants with a prior diagnosis of osteoporosis or Paget's disease ($P = .008$; n = 3476; Supplementary Table II, online only, sensitivity analysis I). The finding was similar in the same participants when adjustment for the history of smoking was replaced with pack-years smoked (Supplementary Table II, online only, sensitivity analysis II). Findings were also similar irrespective of the risk factors that were adjusted for when participants were divided into sufficient, high, and deficient vitamin D levels (Supplementary Table II, online only).

A further sensitivity analysis excluding participants with a prior diagnosis of osteoporosis or Paget's disease from the growth cohort (n = 172) suggested that higher deseasonalized 25(OH)D_3 concentration was associated with significantly faster annual AAA growth after adjusting for time, age, initial AAA diameter, history of smoking, and diabetes (0.6 mm/y, 95% CI: 0.3, 0.9; $P = .001$; Supplementary Table III, online only, sensitivity analysis I). Findings were similar when adjustment for the history of smoking was replaced with pack-years smoked (Supplementary Table III, online only, sensitivity analysis II). Findings were also similar irrespective of the risk factors adjusted for when participants were divided into vitamin D sufficient, high, and deficient (Supplementary Table III, online only).

DISCUSSION

The findings suggest that having sufficient 25(OH)D_3 concentration could lower the risk of being diagnosed with AAA. However, exposure to higher concentrations of 25(OH)D_3 in patients with AAA might paradoxically increase the growth rate of small AAAs. This was evident

Table V. Estimates of the association between continuous and categorical deseasonalized 25(OH)D_3 concentration and mean annual abdominal aortic aneurysm (AAA) growth

	Unadjusted model			Adjusted model		
25 (OH)D_3	MD, mm/yr	95% CI	*P*	MD	95% CI	*P*
All participants (n = 310)						
Per 1 SD increase	0.1	0.1-0.1	<.001	0.1	0.0-0.1	<.001
<50 nmol/L	0.0	−0.1 to 0.1	.988	0.0	−0.1 to 0.1	.949
50 to <75 nmol/L			Reference			
≥75 nmol/L	0.1	0.1-0.2	<.001	0.1	0.1-0.2	<.001
Men only (n = 295)						
Per 1 SD increase	0.1	0.0-0.1	<.001	0.1	0.1-0.2	.002
<50 nmol/L	−0.1	−0.3 to 0.2	.654	−0.1	−0.3 to 0.2	.697
50 to <75 nmol/L			Reference			
≥75 nmol/L	0.6	0.3-0.9	<.001	0.6	0.3-0.9	<.001

25 (OH)D_3, 25-Hydroxy vitamin D; *CI*, confidence interval; *IQR*, interquartile range; *MD*, mean difference (reported as change in aortic diameter per year); *SD*, standard deviation.
Adjusted model accounted for time, age, sex, initial AAA diameter, history of smoking, and diabetes. Total growth of participants within the reference group (50 to <75 nmol/L) was 10.8 mm (95% CI: 3.51-18.0) over a median follow-up of 4.2 (IQR: 2.0, 5.8) years.

after adjustment for risk factors for AAA, and findings were consistent in a range of sensitivity analyses.

A recent meta-analysis of observational studies reported a significantly lower circulating 25(OH)D_3 concentration in participants with AAA than those without.[8] However, the inclusions and comparisons were variable. For instance, one study included participants with thoracic aortic aneurysms, and another study compared patients with aortic aneurysm with those with peripheral artery disease (PAD).[22] In the third study, Wong et al[7] reported that serum 25(OH)D_3 concentration was lower in patients with large AA than those with small AAA and was inversely associated with AAA diameter. On the basis of the findings of their study, Wong et al[7] proposed that vitamin D insufficiency was a risk factor for faster AAA progression rather than formation. This hypothesis was not, however, specifically examined. The current findings were consistent with the previous meta-analysis of observational studies.[8] Nevertheless, in all observational studies confounding or reverse causality cannot be excluded. For example, comorbidities that increase risk of AAA may be associated with reduced sun exposure, leading to lower 25(OH)D_3 concentration. Randomized controlled trials or Mendelian randomization studies might help to elucidate the causality of the observed association.

An unmet clinical need is the knowledge about the role of modifiable risk factors or treatment to limit AAA growth. We found no evidence that low 25(OH)D_3 concentration was associated with faster AAA growth. In contrast, 1 SD higher 25(OH)D_3 concentration was associated with significantly faster AAA growth, which was consistent with the analysis of 25(OH)D_3 as a categorical variable. There could be three possible reasons for this finding. First, vitamin D plays a key role in calcium metabolism and, in higher concentrations, has been associated with vascular calcification,[23] which is implicated in AAA development, although conflicting findings exist from animal[5,24] and human studies.[25-27] It is also likely that vitamin D metabolism and aortic and bone metabolism have distinct and complex pathways in mice and people. Secondly, inadequate control for confounders, particularly atherosclerosis, could influence the observed association.[28] Although athero-occlusive disease is a risk factor for AAA diagnosis, it has been associated with slower AAA growth.[29] Patients with PAD or coronary heart disease may have lower 25(OH)D_3 concentration due to less sunlight exposure, and the association between coronary heart disease and vitamin D deficiency independent of cardiovascular risk factors has been established.[30] PAD has been shown to be associated with slower AAA growth.[29,31] Importantly, a history of hypertension was substantially higher in the AAA diagnosis data set (62.5%) than in the AAA growth subset (49.8%) in the current study, suggesting that the findings may not be representative of the broader AAA population. Thirdly, there could be a complex bimodal relationship of 25(OH)D_3 concentration with AAA diagnosis and growth. A similar pattern has been shown in other conditions.[32,33] Specifically, vascular calcification has been reported in hypervitaminosis D.[34] The multimodal relationship hypothesis could explain the contrasting association of 25(OH)D_3 concentration with AAA diagnosis, where it is protective, and growth pattern where it seems to be harmful. These findings need to be replicated with more mechanistic insights before they are used to inform clinical decision-making.

This study should be considered in light of several limitations. First, Australia is geographically large, and the intensity of ambient ultraviolet radiation varies between

regions, with greater variability in winter than in summer. We accounted for the potential influence of this on circulating 25(OH)D_3 concentration by recruiting cases and controls residing in the same state and analyzing the Queensland and WA participants' results separately. We also accounted for seasonal variations by using deseasonalized 25(OH)D_3 concentration calculated with a validated method.[17] However, it was not possible to completely rule out residual confounding, and other key factors such as sample collection at different time periods, and variability in management of contemporary and historical population. It is possible that changes in risk factor management over time may have influenced the findings. Secondly, the absence of AAA in Queensland control participants was not confirmed by ultrasound imaging. Based on the WA data, approximately 5.6% of participants screened from the community had AAA. Therefore, it is likely that some participants in the Queensland control may have undiagnosed AAA. Thirdly, because the WA cohort included only men, women were under-represented in this analysis. Future studies focused on women with AAA are needed. Lastly, although AAA growth was significantly faster in participants with high compared with sufficient vitamin D levels, this finding needs to be validated and may not be clinically significant given the small differences identified.[35]

CONCLUSIONS

Higher 25(OH)D_3 concentration was paradoxically associated with reduced odds of diagnosis of aneurysm but faster AAA growth. Further research is needed to resolve these conflicting findings.

AUTHOR CONTRIBUTIONS

Conception and design: ST, RN, JG
Analysis and interpretation: ST, RN, MW, JM, MC, JG
Data collection: RN, MW, BY, PN, LF, GH, JJ, FQ, MC, JG
Writing the article: ST
Critical revision of the article: ST, RN, MW, JM, BY, PN, LF, GH, JJ, FQ, MC, JG
Final approval of the article: ST, RN, MW, JM, BY, PN, LF, GH, JJ, FQ, MC, JG
Statistical analysis: ST, RN, MW
Obtained funding: RN, MW, JM, BY, PN, LF, GH, JG
Overall responsibility: ST, RN, MW, JM, BY, PN, LF, GH, JJ, FQ, MC, JG

DISCLOSURES

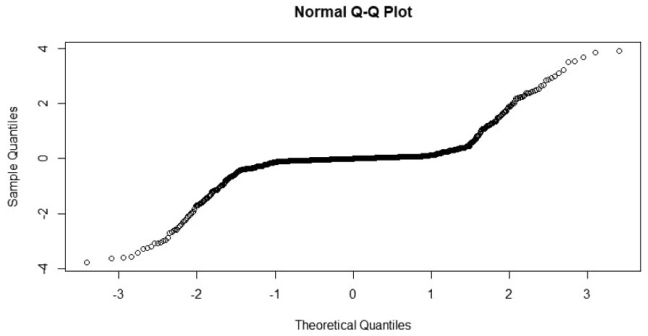
None.

REFERENCES

1. Chaikof EL, Dalman RL, Eskandari MK, et al. The Society for Vascular Surgery practice guidelines on the care of patients with an abdominal aortic aneurysm. *J Vasc Surg*. 2018;67:2–77.e2.
2. Sampson UKA, Norman PE, Fowkes FGR, et al. Global and regional burden of aortic dissection and aneurysms: mortality trends in 21 world regions, 1990 to 2010. *Global Heart*. 2014;9:171–180.e10.
3. Golledge J, Moxon JV, Singh TP, Bown MJ, Mani K, Wanhainen A. Lack of an effective drug therapy for abdominal aortic aneurysm. *J Intern Med*. 2020;288:6–22.
4. Wanhainen A, Verzini F, Van Herzeele I, et al. Editor's choice—European Society for Vascular Surgery (ESVS) 2019 clinical practice guidelines on the management of abdominal aorto-iliac artery aneurysms. *Eur J Vasc Endovasc Surg*. 2019;57:8–93.
5. Nsengiyumva V, Krishna SM, Moran CS, et al. Vitamin D deficiency promotes large rupture-prone abdominal aortic aneurysms and cholecalciferol supplementation limits progression of aneurysms in a mouse model. *Clin Sci (Lond)*. 2020;134:2521–2534.
6. Martorell S, Hueso L, Gonzalez-Navarro H, Collado A, Sanz MJ, Piqueras L. Vitamin D receptor activation reduces angiotensin-II-induced dissecting abdominal aortic aneurysm in apolipoprotein E-Knockout mice. *Arterioscler Thromb Vasc Biol*. 2016;36:1587–1597.
7. Wong YY, Flicker L, Yeap BB, McCaul KA, Hankey GJ, Norman PE. Is hypovitaminosis D associated with abdominal aortic aneurysm, and is there a dose-response relationship? *Eur J Vasc Endovasc Surg*. 2013;45:657–664.
8. Takagi H, Umemoto T, Alice (All-Literature Investigation of Cardiovascular Evidence) group. Vitamins and abdominal aortic aneurysm. *Int Angiol*. 2017;36:21–30.
9. Lutsey PL, Rooney MR, Folsom AR, Michos ED, Alonso A, Tang W. Markers of vitamin D metabolism and incidence of clinically diagnosed abdominal aortic aneurysm: the Atherosclerosis Risk in Communities Study. *Vasc Med*. 2018;23:253–260.
10. Golledge J, Cronin O, Iyer V, Bradshaw B, Moxon JV, Cunningham MA. Body mass index is inversely associated with mortality in patients with peripheral vascular disease. *Atherosclerosis*. 2013;229:549–555.
11. Neale RE, Armstrong BK, Baxter C, et al. The D-Health Trial: a randomized trial of vitamin D for prevention of mortality and cancer. *Contemp Clin Trials*. 2016;48:83–90.
12. Waterhouse M, English DR, Armstrong BK, et al. A randomized placebo-controlled trial of vitamin D supplementation for reduction of mortality and cancer: statistical analysis plan for the D-Health Trial. *Contemp Clin Trials Commun*. 2019;14:100333.
13. Norman PE, Flicker L, Almeida OP, Hankey GJ, Hyde Z, Jamrozik K. Cohort profile: the Health in men study (HIMS). *Int J Epidemiol*. 2008;38:48–52.
14. Golledge J, Moxon J, Pinchbeck J, et al. Association between metformin prescription and growth rates of abdominal aortic aneurysms. *Br J Surg*. 2017;104:1486–1493.
15. Matthews EO, Pinchbeck J, Elmore K, Jones RE, Moxon JV, Golledge J. The reproducibility of measuring maximum abdominal aortic aneurysm diameter from ultrasound images. *Ultrasound J*. 2021;13:13.
16. Clarke MW, Tuckey RC, Gorman S, Holt B, Hart PH. Optimized 25-hydroxyvitamin D analysis using liquid-liquid extraction with 2D separation with LC/MS/MS detection, provides superior precision compared to conventional assays. *Metabolomics*. 2013;9:1031–1040.
17. Waterhouse M, Baxter C, Duarte Romero B, et al. Predicting deseasonalised serum 25 hydroxy vitamin D concentrations in the D-Health Trial: an analysis using boosted regression trees. *Contemp Clin Trials*. 2021;104:106347.
18. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Relationship of the type of tobacco and inhalation pattern to pulmonary and total mortality. *Eur Respir J*. 1992;5:1111–1117.
19. Hartigan JA, Hartigan PM. The dip test of unimodality. *Ann Stat*. 1985;13:70–84.
20. Pinheiro J, Bates D, DebRoy S, Sarkar D. nlme: linear and nonlinear mixed effects models. Accessed June 13, 2023. https://CRAN.R-project.org/package=nlme.
21. Wickham H. *ggokit2: Elegant graphics for data analysis*. Springer-Verlag; 2016.
22. van de Luijtgaarden KM, Voute MT, Hoeks SE, et al. Vitamin D deficiency may be an independent risk factor for arterial disease. *Eur J Vasc Endovasc Surg*. 2012;44:301–306.
23. Zittermann A, Schleithoff SS, Koerfer R. Vitamin D and vascular calcification. *Curr Opin Lipidol*. 2007;18:41–46.
24. Moran CS, McCann M, Karan M, Norman P, Ketheesan N, Golledge J. Association of osteoprotegerin with human abdominal aortic aneurysm progression. *Circulation*. 2005;111:3119–3125.

25. Nieuwland AJ, Kokje VBC, Koning OH, et al. Activation of the vitamin D receptor selectively interferes with calcineurin-mediated inflammation: a clinical evaluation in the abdominal aortic aneurysm. *Lab Invest*. 2016;96:784—790.
26. Wijenayaka AR, Prideaux M, Yang D, et al. Early response of the human SOST gene to stimulation by 1alpha,25-dihydroxyvitamin D3. *J Steroid Biochem Mol Biol*. 2016;164:369—373.
27. St John HC, Hansen SJ, Pike JW. Analysis of SOST expression using large minigenes reveals the MEF2C binding site in the evolutionarily conserved region (ECR5) enhancer mediates forskolin, but not 1,25-dihydroxyvitamin D3 or TGFbeta1 responsiveness. *J Steroid Biochem Mol Biol*. 2016;164:277—280.
28. Golledge J, Norman PE. Atherosclerosis and abdominal aortic aneurysm: cause, response, or common risk factors? *Arterioscler Thromb Vasc Biol*. 2010;30:1075—1077.
29. Matthews EO, Moxon JV, Singh TP, et al. Athero-occlusive disease appears to be associated with slower abdominal aortic aneurysm growth: an exploratory analysis of the TEDY trial. *Eur J Vasc Endovasc Surg*. 2022;63:632—640.
30. Siadat ZD, Kiani K, Sadeghi M, Shariat AS, Farajzadegan Z, Kheirmand M. Association of vitamin D deficiency and coronary artery disease with cardiovascular risk factors. *J Res Med Sci*. 2012;17:1052—1055.
31. Takagi H, Umemoto T, Group A. Association of peripheral artery disease with abdominal aortic aneurysm growth. *J Vasc Surg*. 2016;64:506—513.
32. Shroff R, Egerton M, Bridel M, et al. A bimodal association of vitamin D levels and vascular disease in children on dialysis. *J Am Soc Nephrol*. 2008;19:1239—1246.
33. Lim JH, Ravikumar S, Wang YM, et al. Bimodal influence of vitamin D in host response to systemic Candida infection-vitamin D dose matters. *J Infect Dis*. 2015;212:635—644.
34. Drüeke TB, Massy ZA. Role of vitamin D in vascular calcification: bad guy or good guy? *Nephrol Dial Transplant*. 2012;27:1704—1707.
35. Kraemer HC, Mintz J, Noda A, Tinklenberg J, Yesavage JA. Caution regarding the use of pilot studies to guide power calculations for study proposals. *Arch Gen Psychiatr*. 2006;63:484—489.

Submitted Feb 25, 2024; accepted May 14, 2024.



Supplementary Fig (online only). Quantile-quantile (Q-Q) plot showing an overdispersed distribution in the best-fit model, suggesting the presence of multimodality.

Supplementary Table I (online only). Month-specific mean values of raw and deseasonalized 25(OH)D_3 concentrations of all the included participants

Month of blood collection	Raw 25(OH)D_3 concentrations (mean ± SD)	Deseasonalized 25(OH)D_3 concentrations (mean ± SD)	Raw 25(OH)D_3 concentrations (mean ± SD)	Deseasonalized 25(OH)D_3 concentrations (mean ± SD)
	Controls (n = 4043)		Cases (n = 630)	
January	86.62 ± 22.30	82.37 ± 22.30	82.45 ± 20.90	78.2 ± 20.90
February	75.58 ± 21.43	68.58 ± 21.43	80.55 ± 25.57	73.55 ± 25.57
March	79.28 ± 24.98	71.49 ± 24.98	85.43 ± 26.76	77.65 ± 26.76
April	76.48 ± 22.54	70.09 ± 22.54	74.67 ± 19.32	68.28 ± 19.32
May	76.23 ± 28.63	73.03 ± 28.63	76.53 ± 21.30	73.34 ± 21.30
June	66.72 ± 21.04	67.66 ± 21.04	66.07 ± 26.34	67.02 ± 26.34
July	65.53 ± 23.18	70.45 ± 23.18	71.47 ± 23.12	76.39 ± 23.12
August	61.42 ± 23.01	69.09 ± 23.01	70.63 ± 25.19	78.3 ± 25.19
September	63.79 ± 23.54	72.24 ± 23.54	67.21 ± 28.22	75.67 ± 28.22
October	62.82 ± 21.10	69.89 ± 21.10	73.53 ± 27.15	80.59 ± 27.15
November	65.21 ± 18.94	69.08 ± 18.94	72.39 ± 22.29	76.26 ± 22.29
December	66.83 ± 20.57	66.56 ± 20.57	78.73 ± 25.91	78.46 ± 25.91

25 (OH)D_3, 25-Hydroxy vitamin D; *SD*, standard deviation.

Supplementary Table II (online only). Sensitivity analyses of the association between continuous and categorical deseasonalized 25(OH)D_3 concentration and abdominal aortic aneurysm (AAA) diagnosis in Western Australian participants

25 (OH)D_3	OR	95% CI	*P*
Sensitivity analysis I (n = 3476)			
Per 1 SD increase	0.81	0.69-0.94	.008
<50 nmol/L	1.24	0.85-1.78	.251
50 to <75 nmol/L	Reference		
≥75 nmol/L	0.76	0.53-1.08	.135
Sensitivity analysis II (n = 3476)			
Per 1 SD increase	0.81	0.69-0.95	.010
<50 nmol/L	1.27	0.88-1.83	.198
50 to <75 nmol/L	Reference		
≥75 nmol/L	0.80	0.55-1.13	.207

25 (OH)D_3, 25-Hydroxy vitamin D; *CI*, confidence interval; *OR*, odds ratio; *SD*, standard deviation.
Sensitivity analysis I adjusted for age, history of smoking, hypertension, and diabetes. Sensitivity analysis II adjusted for age, pack-years smoked, systolic blood pressure, and diabetes. Both analyses excluded participants with a prior diagnosis of osteoporosis or Paget's disease and included the same participants.

Supplementary Table III (online only). Sensitivity analysis of the association between continuous and categorical deseasonalized 25(OH)D_3 concentration and abdominal aortic aneurysm (AAA) growth in Western Australian participants

25 (OH)D_3	MD (mm/y)	95% CI	*P*
Sensitivity analysis I (n = 172)			
Per 1 SD increase	0.6	0.3 to 0.9	.001
<50 nmol/L	−1.5	−2.2 to −0.7	<.001
50 to <75 nmol/L	Reference		
≥75 nmol/L	0.0	−0.7 to 0.8	.985
Sensitivity analysis II (n = 172)			
Per 1 SD increase	0.6	0.3-0.9	<.001
<50 nmol/L	−1.7	−2.4 to 0.9	<.001
50 to <75 nmol/L	Reference		
≥75 nmol/L	0.0	−0.8 to 0.7	.911

25(OH)D_3, 25-Hydroxy vitamin D; *CI*, confidence interval; *MD*, mean difference (reported as change in aortic diameter per year); *SD*, standard deviation.
Sensitivity analysis I adjusted for time, age, initial AAA diameter, history of smoking and diabetes. Sensitivity analysis II adjusted for time, age, initial AAA diameter, pack-years, and diabetes in the same participants. Both analyses excluded participants with a prior diagnosis of osteoporosis or Paget's disease and included the same participants.