Is Hypovitaminosis D Associated with Abdominal Aortic Aneurysm, and is There a Dose—response Relationship?

Y.Y.E. Wong a,b,c,*, L. Flicker a,b,c, B.B. Yeap b,d, K.A. McCaul b, G.J. Hankey e, P.E. Norman f

a Western Australian Centre for Health and Ageing, Centre for Medical Research, Western Australian Institute for Medical Research, Perth, Australia
b School of Medicine and Pharmacology, University of Western Australia, Perth, Australia
c Department of Geriatric Medicine, Royal Perth Hospital, Perth, Australia
d Department of Endocrinology and Diabetes, Fremantle Hospital, Perth, Australia
e Department of Neurology, Royal Perth Hospital, Perth, Australia
f School of Surgery, University of Western Australia, Perth, Australia

WHAT THIS PAPER ADDS
The relationship between vitamin D status and abdominal aortic aneurysm (AAA) has not been clearly defined. We conducted an observational study and found an inverse relationship between 25-hydroxyvitamin D (25(OH)D) and the presence of AAA in community-dwelling older men. There is also a negative dose—response association between 25(OH)D and the size of AAA, suggesting a role of vitamin D in the severity of aneurysmal arterial disease. To our knowledge, this is the first study to demonstrate an independent relationship between hypovitaminosis D with the presence and size of AAA in older men. Further research is needed to clarify the mechanisms underlying these associations.

Objective: This study aims to investigate the association between plasma 25-hydroxyvitamin D (25(OH)D) concentrations with the presence of abdominal aortic aneurysm (AAA) and aortic diameter.

Design: An observational study of 4233 community-dwelling men aged 70–88 years, who participated in a randomised controlled trial of screening for AAA.

Methods: Infrarenal aortic diameter measured by ultrasound and 25(OH)D by immunoassay.

Results: A total of 311 men (7.4%) with AAA (defined as aortic diameter ≥30 mm) comprised the study. Multivariable models were adjusted for age, smoking, cardiovascular disease, hypertension, diabetes, dyslipidaemia, body mass index and serum creatinine concentration. Amongst men with the lowest 25(OH)D quartile of values compared with the highest quartile, the adjusted odds ratio of having an AAA increased in a graded fashion from 1.23 (95% confidence interval (CI) 0.87–1.73) for AAA ≥30 mm to 5.42 (95% CI 1.85–15.88) for AAA ≥40 mm. Similarly, there was a dose—response relationship between 25(OH)D concentrations and the size of the AAA: every 10-nmol l⁻¹ decrease in 25(OH)D levels was associated with 0.49 mm (95% CI 0.11–0.87) increase in mean aortic diameter.

Conclusions: Low vitamin D status is associated with the presence of larger AAA in older men, and there is a graded inverse relationship between 25(OH)D concentrations and AAA diameter. Further research is needed to clarify the mechanisms underlying these associations.

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Vitamin D and its metabolites are known to have significant roles in the maintenance of calcium and bone homeostasis. In recent years, they have also been extensively researched for their pleiotropic effects on biological processes other than skeletal health. Epidemiological studies have suggested that hypovitaminosis D might be a contributory factor to cardiovascular disease (CVD) including ischaemic heart disease, peripheral arterial disease (PAD) and stroke.¹ It is also linked to major traditional CVD risk factors, such as hypertension, dyslipidaemia and obesity,² as well as CVD mortality.³
Postulated mechanisms for these presumed cardiovascular effects include endothelial dysfunction, inflammation, reduced vessel compliance, detrimental effects via bone proteins such as osteoprotegerin as well as dysregulation of the renin-angiotensin system. On the other hand, a recent systematic review and meta-analysis of vitamin D intervention trials failed to demonstrate a significant reduction in CVD events with vitamin D supplementation, thus implying that hypovitaminosis D might be an epiphenomenon, rather than as an aetiologically factor for CVD.

Aneurysmal arterial disease is pathologically characterised by extensive disruption of the extracellular matrix with the loss of medial elastin and consequent reduction in tensile strength. This results in vascular wall weakening and progressive localised dilatation. Abdominal aortic aneurysm (AAA) and occlusive CVD share many risk factors, including age, male gender, genetic predisposition, smoking, hypertension, hypercholesterolaemia and obesity. As AAA is also independently associated with a higher risk of incident CVD events and mortality, it has been traditionally considered as a manifestation of atherosclerosis. However, there are important differences between aneurysmal and occlusive CVD suggesting disparate pathogenesis. The most notable difference is the inverse association between diabetes and AAA.

Vitamin D influences a range of molecular pathways of potential relevance to the pathogenesis of AAA. As the relationship between vitamin D status and AAA is unknown, we sought to determine whether there is any relationship between plasma concentration of vitamin D and both the presence and diameter of AAA. This was undertaken in a large cohort of men aged 70–88 years.

SUBJECTS AND METHODS

Study design and participants

We conducted a cross-sectional observational study of participants from the Health in Men Study (HIMS), which has been described in detail elsewhere. In brief, approximately 40,000 men aged 65–83 years were randomised to the screening and control arms of the trial of screening for AAA. A total of 12,203 men participated in screening and completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001–2004, 5585 men responded to the second phase of this study (HIMS Wave 2) and blood samples were collected from 4249 of them. The Human Research Ethics Committee of the University of Western Australia approved the protocol for HIMS and informed consent was obtained from the participants.

Outcome of interest

The abdominal aortic diameter was measured during HIMS Wave 1. The greatest transverse and antero-posterior diameter of the infrarenal aorta was measured using a Toshiba Capasee ultrasound machine with a 3.75-MHz probe (Toshiba Australia, North Ryde, New South Wales, Australia). The reproducibility of these ultrasound measurements has been previously reported. An AAA was considered present if the abdominal aortic diameter was 30 mm or greater.

Explanatory variables

Using a combination of data collected at Waves 1 and 2, the following variables were available: age, smoking status (current, former or never smoker during Wave 2), physical activity (≥150 min of moderate-to-vigorous exercise in a usual week during Wave 1), CVD (self-reported history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke during Wave 1 or 2), hypertension (self-reported diagnosis, or use of anti-hypertensive medications or measured average blood pressure > 140/90 mmHg), diabetes (self-reported diagnosis, or use of glucose-lowering medication, or a fasting glucose of ≥7 mmol l⁻¹ or non-fasting glucose ≥11 mmol l⁻¹) and dyslipidaemia (self-reported diagnosis, or use of lipid-lowering medication, or fasting low-density lipoprotein ≥3.4 mmol l⁻¹, high-density lipoprotein <0.9 mmol l⁻¹, triglycerides ≥1.8 mmol l⁻¹ or total cholesterol ≥5.5 mmol l⁻¹). Height, weight and waist circumference were measured during Wave 2 in accordance with guidelines of the International Society for the Advancement of Kinanthropometry. Body mass index (BMI) was calculated from height and weight in kg m⁻².

Validation of CVD variables

In addition to data from the questionnaire, the Western Australian Data Linkage System (WADLS) was used to identify men with any admissions to hospital for CVD by the end of Wave 2 screening. This system links together data from the state cancer registry, death registry and hospital morbidity data system. The admissions were identified using the relevant diagnoses and procedure codes from the International Classification of Diseases (ICD). The predictive utility of CVD for all-cause mortality was tested in our cohort by using mortality records up to 31 December 2010.

Biochemical analyses

Blood samples were collected during Wave 2 between 0800 and 1030. Plasma was separated from the blood samples within 1 h of collection and stored at −80 °C until assayed. Plasma 25-hydroxyvitamin D [25(OH)D], an established marker of vitamin D status, was measured using the automated DiaSorin ‘LIAISON 25(OH)D’ immunoassay, which was carried out on archived serum in a series of runs performed between 2011 and 2012. The interassay coefficient of variation was 13.2% at 37.9 nmol l⁻¹ and 11.3% at 131 nmol l⁻¹. Serum high-sensitivity C-reactive protein (hsCRP) was measured with assay on a BNII analyser (Dade Behring, Birmingham, UK). Serum creatinine, glucose, cholesterol, low-density lipoprotein and triglycerides were measured with a Roche Hitachi 917 analyser (Roche Diagnostics, Basel, Switzerland).
Statistical analysis
Data were analysed using Stata release 11.1 (Stata Corp, College Station, TX, USA). Descriptive statistics were calculated for the demographic, lifestyle and clinical variables according to the presence or absence of AAA. A Cox proportional hazards model was used to test the association between CVD and mortality. Adjustments were made for age, smoking, hypertension, diabetes, dyslipidaemia, BMI and creatinine. The association between 25(OH)D and AAA was investigated in three different ways: according to 25(OH)D quartiles of values (using the highest quartile as reference), per 10-nmol l\(^{-1}\) decrease in 25(OH)D concentration and by halving of 25(OH)D concentration (by dividing the natural logarithm of 25(OH)D by the natural logarithm of 0.5). To investigate the relationship between 25(OH)D with the presence of AAA, logistic regression analyses were performed with 25(OH)D modelled as continuous and categorical variables. The associations were explored for the presence of any AAA (aortic diameter ≥30 mm) and larger AAAs (diameter ≥35 mm and ≥40 mm, respectively). To determine the relationship between 25(OH)D and aortic diameter, linear regression was used with the aortic diameter modelled as a continuous variable in aneurysmal (aortic diameter ≥30 mm) and non-aneurysmal (<30 mm) ranges. Adjustments were made for age, smoking, CVD, diabetes, dyslipidaemia, BMI and serum creatinine as potential confounders.

The results were reported as odds ratios (ORs) with 95% confidence intervals (95% CI). *P*-values <0.05 were considered statistically significant.

RESULTS
A flow chart detailing disposition of the study participants is shown in Fig. 1. Men who had died prior to the Wave 2 follow-up or did not attend due to various reasons were older in age (*P* < 0.001), more likely to be smokers (*P* < 0.001) and self-report a history of CVD (*P* = 0.025), hypertension (*P* < 0.001) and diabetes (*P* < 0.001) during Wave 1, in comparison to those men who subsequently responded in Wave 2. The demographic, clinical and biochemical characteristics of men with and without AAA are shown in Table 1. A total of 4233 men, aged 70–88 years, had complete data for 25(OH)D levels, aortic diameter and co-morbidities. The mean (±standard deviation (SD)) 25(OH)D concentration for the cohort was 68.3 ± 23.3 nmol l\(^{-1}\) and the quartiles of values correspond to 10.0–52.8 (median 42.3), 52.9–67.3 (median 60.3), 67.4–81.6 (median 73.8) and 81.7–238.4 nmol l\(^{-1}\) (median 93.7 nmol l\(^{-1}\)), respectively. As many as 311 men (7.4%) had an AAA (aortic diameter ≥30 mm), 120 men (2.8%) had AAA ≥35 mm and 66 men (1.6%) had AAA ≥40 mm. The mean (±SD) aortic diameter for the cohort was 22.8 ± 4.9 mm (range 15.9–79.2 mm).

Predictive utility of CVD for all-cause mortality
In the multivariable Cox proportional hazards model, CVD predicted all-cause mortality (hazard ratio (HR) 1.53, 95% CI 1.36–1.73).

Association of 25(OH)D with AAA
In multivariable logistic regression analysis (Table 2), the adjusted OR of having an AAA for men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.23 (95% CI 0.87–1.73). This association became statistically significant and stronger in a graded fashion for larger AAAs, with an OR of 2.02 (95% CI 1.13–3.61) for AAA ≥35 mm and an OR of 5.42 (95% CI 1.85–15.88) for AAA ≥40 mm. When 25(OH)D was modelled as continuous variables, there was a graded increase in association with larger AAAs: for every 10-nmol l\(^{-1}\) decrease in 25(OH)D concentration, the odds were increased by 14% (OR 1.14, 95% CI 1.04–1.24) for having AAA ≥35 mm and by 23% (OR 1.23, 95% CI 1.08–1.39) for having AAA ≥40 mm. Fig. 2 demonstrates the OR of having an AAA ≥40 mm with changing 25(OH)D levels.

Association of 25(OH)D with aortic diameter
We explored the association of 25(OH)D with abdominal aortic diameter by stratifying the aortic diameter into aneurysmal (≥30 mm) and non-aneurysmal (<30 mm) ranges (Table 3). Adjustments were made for age, smoking, CVD, hypertension, diabetes, dyslipidaemia, BMI and serum creatinine. There was no statistically significant association between 25(OH)D and aortic diameters in the non-aneurysmal range. For men with aortic diameters ≥30 mm, every 10-nmol l\(^{-1}\) decrease in 25(OH)D concentration was associated with 0.49 (95% CI 0.11–0.87) increase in mean aortic diameter. For those men with the lowest 25(OH)D quartile of values compared with the highest quartile, there was an associated 3.06 (95% CI 0.70–5.41) increment in aortic diameter. The associations between 25(OH)D and aortic diameter in the aneurysmal range persisted when aortic diameter was log-transformed. Every 10-nmol l\(^{-1}\) decrease in 25(OH)D concentration was associated with 0.01 mm (95% CI 0.00–0.02) increase in log-transformed aortic diameter. For those men with the lowest 25(OH)D quartile of values compared with the highest quartile, there was an associated 0.08 mm (95% CI 0.02–0.13) increment in log-transformed aortic diameter.

Subgroup analyses
Vitamin D supplementation might influence 25(OH)D concentration and these data were available at the Wave 2 assessment. Ten men with AAA and 90 men without AAA had reported taking these supplements (*P* = 0.303). We performed a sensitivity analysis of the association between 25(OH)D and AAA by excluding men taking supplements. The effect estimate for the presence of an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was not significantly affected (OR 1.18, 95% CI 0.84–1.67). For AAA ≥35 mm and AAA ≥40 mm, the ORs were 1.95 (95% CI 1.08–3.50) and 5.56 (95% CI 1.90–16.32), respectively. Additional adjustments for physical activity, waist circumference and hsCRP also did not appreciably alter the association of 25(OH)D with AAA (data not shown).
When seasonality was added to the multivariable logistic regression models, the odds of having an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.27 (95% CI 0.90–1.81). For AAA ≥ 35 mm and AAA ≥ 40 mm, the ORs were 1.92 (95% CI 1.07–3.47) and 4.69 (95% CI 1.59–13.85), respectively. For blood samples collected during the summer/autumn and winter/spring calendar periods, the lowest quartile of 25(OH)D values corresponded to 58.8 and 48.0 nmol l⁻¹, respectively. When multivariable logistic regression analysis was performed using 25(OH)D collected during summer/autumn, the odds of having an AAA in men with the lowest 25(OH)D quartile of values compared with the highest quartile was 1.27 (95% CI 0.88–1.83). When this was repeated for 25(OH)D collected during winter/spring, the OR remained similar (OR 1.25, 95% CI 0.90–1.73). When the analyses were repeated for AAA ≥ 35 and AAA ≥ 40 mm stratified by the season of blood sample collection, the associations persisted.

**DISCUSSION**

In this population-based sample of older men, there is an association between low 25(OH)D concentration and the presence of larger AAA, which is independent of traditional cardiovascular risk factors, and occurs in a graded fashion with increasing sizes of AAA. In men with AAA, 25(OH)D concentration is also inversely correlated with abdominal aortic diameter in a dose–response relationship.

The strengths of this study include the large sample size of well-characterised, population-based community-dwelling older men. Measurement of the aortic diameter was performed in a standardised procedure using ultrasound. We were able to comprehensively adjust for
potential confounders in our multivariable analyses, including age, smoking, CVD and risk factors that have established associations with both vitamin D status and AAA development. We acknowledge several limitations in this study. The observational design of our current study and the fact that blood samples were obtained from the participants several years (5.7 ± 0.9 years) after baseline aortic diameter measurements preclude determination of causality over time. We did not have data on calcium or parathyroid hormone, which can influence vitamin D metabolism. Aortic diameters in our cohort were likely to have increased by a small margin between the period of baseline measurement and blood sampling. However, we have assumed in this study that the small number of interval cases of AAA would not have substantially altered the results or introduced any bias. Those men who had responded for assessment during Wave 2 were younger in age (P < 0.001), more physically active (P < 0.001) and had less self-reported co-morbidities (P < 0.001) during Wave 1 compared to the non-respondents of the follow-up study. The self-selection of participants suggests a possible ‘healthy survivor’ effect, which might have biased our findings towards higher 25(OH)D concentrations in our cohort compared to the non-respondents. This could have moved the results of our study towards a null hypothesis and led to an underestimation of the association between low 25(OH)D with AAA. Our findings were therefore conservative. The study was limited to men and we were therefore unable to generalise our findings to women, although the prevalence of AAA is about five times greater in men than women. To our knowledge, this is the first study to demonstrate an independent relationship between low vitamin D status with the presence and size of AAA in older men. Van de Luijtgaarden et al. have recently reported on the vitamin D status in 236 patients with either thoracic or abdominal aneurysm. Although 9.7% of these patients with aortic aneurysm had vitamin D levels ≤25 nmol l⁻¹.

Table 2. Multivariable logistic regression models b evaluating the association of 25-hydroxyvitamin D [25(OH)D] with the presence of abdominal aortic aneurysm (AAA) in community-dwelling older men.

<table>
<thead>
<tr>
<th>25(OH)D quartile</th>
<th>Or 95% CI</th>
<th>Or 95% CI</th>
<th>Or 95% CI</th>
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<tbody>
<tr>
<td>≥30 mm (n = 311)</td>
<td>1.03 0.98 to 1.09</td>
<td>1.14 1.04 to 1.24</td>
<td>1.23 1.08 to 1.39</td>
</tr>
<tr>
<td>≥35 mm (n = 120)</td>
<td>1.16 0.92 to 1.44</td>
<td>1.60 1.15 to 2.23</td>
<td>1.97 1.28 to 3.02</td>
</tr>
<tr>
<td>≥40 mm (n = 66)</td>
<td>1.23 0.87 to 1.73</td>
<td>2.02 1.13 to 3.61</td>
<td>5.42 1.85 to 15.88</td>
</tr>
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Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval. The quartiles for 25(OH)D corresponded to values of: 10.0–52.8 nmol/l (median 42.7 nmol/l), 52.9–67.3 (median 60.3 nmol/l), 67.4–81.6 nmol/l (median 73.8 nmol/l) and 81.7–238.4 nmol/l (median 93.7 nmol/l), respectively.

a Odds ratio per 10-nmol/l decrease in 25(OH)D concentration.

b Adjusted for age, smoking, cardiovascular disease, hypertension, dyslipidemia, diabetes, body mass index and serum creatinine.
Abbreviation: 95% CI, 95% confidence interval. The quartiles for 25(OH)D corresponded to values of: 10.0–25.8 nmol/l (median 42.7 nmol/l), 52.9–67.3 (median 60.3 nmol/l), 67.4–81.6 nmol/l (median 73.8 nmol/l) and 81.7–238.4 nmol/l (median 93.7 nmol/l), respectively.

a Adjusted for age, smoking, cardiovascular disease, hypertension, diabetes, dyslipidemia, body mass index and serum creatinine.

Our prevalence of AAA was 7.4%, which was relatively high in comparison to those derived from other large AAA screening programmes. There are a number of possible reasons: men aged up to 83 years, rather than the usual 65–74 years, were included in our study (aortic diameter increases with age), and the fact that measurement of aortic diameter was performed using ‘outer to outer’ wall (this gives the largest possible diameter). The relatively lower proportions of larger AAAs may be explained by these cases being offered surgery prior to the blood-testing phase of the study. The inverse relationship between diabetes and AAA has been widely reported, albeit inconclusively, due to statistically non-significant findings in some studies. Although we have found a positive association between AAA and the presence of diabetes by the end of Wave 2 screening, a previous analysis using data in Wave 1 reported an inverse correlation between serum glucose and aortic diameter in non-diabetic men. Despite demonstrating seasonal variation in plasma 25(OH)D concentrations, this has not altered the association between low vitamin D status and the presence of larger AAA in our cohort of older men.

In conclusion, we have found an inverse relationship between vitamin D status and the prevalence of larger AAA, irrespective of traditional CVD and associated risk burden, in community-dwelling older men. There is also an inverse dose–response association between 25(OH)D concentrations and the size of AAA, suggesting a role of vitamin D in the severity of aneurysmal arterial disease. Further research is needed to clarify the mechanisms underlying these associations and to explore the feasibility of interventional studies with abdominal aortic diameter as an ‘end’ point.

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**CONFLICT OF INTEREST**

None declared.

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