Title: Serum 25-hydroxyvitamin D deficiency and the 5-year incidence of chronic kidney disease

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ABSTRACT

Background: Low serum 25-hydroxyvitamin D (25(OH)D) levels have been associated with chronic kidney disease (CKD) in cross-sectional studies, however this association has not been studied prospectively in a large, general population-based cohort.

Study Design: Prospective cohort study

Setting & Participants: 6180 adults aged ≥25 years, participating in the baseline and 5-year follow-up phases of the Australian, Diabetes, Obesity and Lifestyle (AusDiab) Study.

Predictor: Serum 25(OH)D levels of < 15ng/ml were considered deficient

Outcomes Measurements: Incident CKD was defined as being negative at baseline but positive after 5-years for (1) impaired eGFR (eGFR <60 ml/min/1.72m^2) or (2) albuminuria (spot urine albumin to creatinine ratio of ≥2.5 mg/mmol (22.1 mg/g) for men and ≥3.5 (30.9 mg/g) for women).

Results: 623 (10.9%) of participants were vitamin D deficient, 161 developed incident impaired eGFR and 222 developed incident albuminuria. In participants with and without vitamin D deficiency, the annual age-standardized incidence was 0.92% (95% CI, 0.56-1.30) and 0.59% (95% CI, 0.51–0.68), respectively for an eGFR <60; and

1.50% (95% CI, 1.06–1.95) and 0.66% (95% CI, 0.56–0.76), respectively for albuminuria. In multivariate regression models, vitamin D deficiency was significantly associated with the 5-year incidence of albuminuria (OR 1.71, 95% CI 1.12-2.61, p=0.01), but not impaired eGFR (OR 0.93, 95% CI 0.53-1.66, p=0.8).

Limitations: The observational nature of the study does not account for unmeasured confounders. Only baseline 25(OH)D level was measured, and therefore may not accurately reflect lifetime levels. Differences in the baseline characteristics of participants who were included, compared to those excluded due to missing data or follow-up may limit the applicability of results to the original AusDiab cohort.

Conclusions: Our prospective cohort study shows that vitamin D deficiency is associated with a higher annual incidence of albuminuria and impaired eGFR, and it independently predicts the 5-year incidence of albuminuria. These associations warrant further exploration in long-term, prospective, clinical trials.

Keywords: vitamin D, albuminuria, chronic kidney disease, glomerular filtration rate, and renal impairment

INTRODUCTION

Vitamin D has a recognized role in human health, and its deficiency has been commonly reported in the general population and chronic kidney disease (CKD) cohorts[1]. Recent research has expanded our understanding of the many effects of vitamin D, beyond its traditional role in regulating bone and mineral metabolism[2].

CKD affects up to 10-15% of the adult population[3]. Proteinuria is an early marker of kidney damage, and an important predictor of CKD progression, cardiovascular outcomes and mortality[4-6]. The decline in renal function is associated with a reduction in calcitriol levels, and numerous cohort studies have linked calcitriol use with decreased morbidity and mortality in dialysis and non-dialysis CKD[7-10]. Experimental data show calcitriol to be a potent inhibitor of the renin-angiotensin system (RAS) and nuclear factor (NF)-κB pathways, which play important roles in the pathogenesis of kidney disease[11,12]. The importance of extra-renal (paracrine and autocrine) calcitriol synthesis by numerous cells, has further implicated low serum 25-hydroxy vitamin D [25(OH)D] levels in the pathogenesis of numerous chronic diseases, including CKD. Consequently low 25(OH)D levels have emerged as a potential risk factor and therapeutic target in CKD[13,14].

In general population studies such as NHANES III, 25(OH)D deficiency has been associated with prevalent albuminuria[15] and progression to end-stage kidney disease (ESKD)[16]. In CKD cohorts, 25(OH)D deficiency has been associated with prevalent albuminuria[17], progression to ESKD[18], and mortality[19]. However these associations

have been derived from cross-sectional or medical record linkage studies and not from large prospective cohorts, thereby limiting the strength of existing evidence. We therefore examined the relationship between 25(OH)D levels and the incidence of albuminuria and impaired eGFR in a large general population cohort of adults, prospectively followed over a 5 year period – the Australian, Diabetes, Obesity and Lifestyle (AusDiab) Study.

METHODS

Study population

AusDiab was a population-based, longitudinal survey of non-institutionalized Australian adults aged 25 years and older. The survey methods and sample collection have been previously described in detail[20-22]. In brief, a stratified cluster sampling method was used, with random selection of clusters based on census collector districts. 6537 of 11,247 (baseline cohort) participants returned for the 5-year follow-up study, and a complete data set was available in 6180 (figure 1). 357 participants had missing data which included: eGFR at 5-years (65), urine albumin-creatinine ratio (UACR) at 5-years (42), 25(OH)D level (24), diabetic assessment (72), blood pressure measurement (7), BMI measurement (29), smoking status (98), baseline UACR (11) and baseline eGFR (9). The AusDiab study was approved by the International Diabetes Institute ethics committee (Melbourne, Australia), and written informed consent was obtained from all participants.

Study Measurements

Details of the biomedical tests have been previously reported[20,21]. In brief, those who attended a testing site at baseline and 5-year follow-up, underwent a physical examination, provided a fasting blood sample, a random spot urine morning collection and a underwent standard 75-g oral glucose tolerance test. Demographic data and information on existing health conditions and health related behaviors were collected using standardized interviewer-administered questionnaires.

Hypertension was defined as a systolic blood pressure of 140 mmHg or greater or a diastolic blood pressure of 90 mmHg or greater. Body mass index (BMI) was calculated from weight and height measurements, with the diagnosis of obesity defined as a BMI of 30 kg/m^2 or greater. Standard World Health Organization criteria for the diagnosis of abnormal glucose metabolism were used. Diabetes was diagnosed on the basis of fasting plasma glucose level of 7.0 mmol/L (126.1mg/dL) or greater, 2-hour plasma glucose level of 11.1 mmol/L (200mg/dl) or greater, or current treatment with insulin or oral hypoglycemic medication. Smoking status was self-reported and participants were classified as either current smoker or non-smoker (ex-smokers and never smoked). Cholesterol and triglycerides were measured on fasting samples. Cardiovascular disease was assessed using self-reported symptoms or past history. Ethnicity was categorized based on the country of birth, with the majority of participants born in Australia and New Zealand, or other English speaking countries. Time of assessment was classified by the season in which venipuncture was performed: summer (December to February), autumn (March to May), winter (June to August) or spring (September to November). The

location of the subjects (urban versus rural) was based upon the classification used by the Australian Bureau of Statistics. The latitude of each blood collection center was determined using the Google® GPS tool and entered as a continuous variable for analysis (range 12°S to 43°S).

Laboratory Methods

Serum 25(OH)D was measured in the entire baseline AusDiab cohort from samples, which were stored at -80ºC using a direct competitive chemiluminescent immunoassay (CLIA) on a Liaison analyzer (DiaSorin Inc. www.diasorin.com) with an inter-assay CV of 7.0% at 18 ng/ml and 6.3% at 37 ng/ml. For participants where fasting serum was not available (n=210), fluoride oxalate plasma was used. Fluoride oxalate plasma was compared with fasting serum samples for the 25(OH)D analysis, both were analysed simultaneously from samples collected from 101 laboratory staff. There was excellent agreement between the two tubes: fluoride oxalate plasma 25(OH)D = 0.97 x serum 25(OH)D + 2.5, r^2=0.89[23].

Serum creatinine was reassessed on all baseline samples using an IDMS aligned enzymatic method (Roche Modular, Roche Diagnostics, www.roche.com); the 5-year samples were re-calibrated using a subgroup of samples (n=389). Urine albumin was measured by rate nephelometry with the Beckman Array (Beckman/Coulter, www.beckmancoulter.com) using fresh urine samples at the time of original collection. Urine creatinine was measured using the modified kinetic Jaffé reaction using an Olympus AU600 autoanalyzer (Olympus Optical, www.olympus.com).

Study Variables

Vitamin D deficiency was defined as a serum 25(OH)D level of <15 ng/ml[16], facilitating comparison to the other large population based study (NHANES). Further analysis was undertaken using 20 ng/ml to define 25(OH)D deficiency, the threshold proposed by the Institute of Medicine.[24]

For subgroup analysis, common clinical cut-off points were used, with 25(OH)D levels of <15, 15-30 and ≥30 ng/ml[25], and the population was divided into quartiles based on the 25(OH)D levels. Incidence of CKD was defined as subjects who were negative at baseline but positive at the 5-year follow-up for each of albuminuria or an impaired eGFR. Incident albuminuria was defined as a UACR of ≥ 2.5 mg/mmol (22.1 mg/g) in males and 3.5 (30.9 mg/g) in females[26]. GFR was estimated using the Chronic Kidney Disease epidemiology (CKD-EPI) equation for white men and women[27]. Incident impaired GFR was defined as an eGFR less than 60 ml/min/1.73m^2, consistent with stage 3 or greater CKD[28].

Statistical analysis

Five-year cumulative incidence for CKD by the 25(OH)D level (deficient or not deficient) was calculated by dividing the total number of incident cases of CKD by the total population free of CKD at baseline. Incidence was standardized to the 1998 Australian population using the direct method.[29] In brief, 5-year cumulative incidence stratified by age and sex strata, were applied to the equivalent age and

strata from the Australian population of 1998 who were free of CKD. The 1998 Australian 'CKD free' population was extracted by applying age and sex specific CKD prevalence estimates from AusDiab to the 1998 population and subtracting the CKD population from the total population to give a CKD free population. The incidence of albuminuria was age and sex standardized, using the same method. Annual incidence (% per year) was calculated from the five-year cumulative incidence by applying the following formula: $(-ln(1-S))/t$; where S is the proportion of new cases over t years and t equals the time of follow-up.

Logistic regression was used to assess the relationship between the two separate CKD variables (albuminuria and impaired eGFR) and the odds of vitamin D deficiency. Four models were constructed: unadjusted (model 1), adjusted for gender and age (model 2), model 2 and determinants of 25(OH)D levels; ethnicity, latitude, season (model 3) and fully adjusted (model 4). Covariates examined included age, gender, BMI, cholesterol, triglycerides, cardiovascular disease, smoking status, ethnicity, diabetic status, location (urban or rural), latitude, season, and blood pressure, baseline UACR and baseline eGFR. Continuous variables used in all regression analyses were not categorized unless otherwise stated. The multivariate model was constructed including covariates that were clinically important or confounders. Interactions between the CKD outcomes and age, sex, diabetes mellitus and ethnicity were assessed. For the albuminuria outcome, the relationship with vitamin D was also modeled as a fractional polynomials function as there was a non-linear relationship between vitamin D level and incident albuminuria. All analyses were conducted using Stata/IC, version 11.1 (Stata Corp, www.stata.com).

RESULTS:

A total of 6180 subjects participating in the follow-up survey had a complete data set for all variables; the baseline characteristics of those who did not have albuminuria or impaired eGFR at baseline (n=5738) are summarized in table 1. The median serum 25(OH)D level was 25 ng/ml (interquartile range 19-31). The proportion of subjects with a level of <15 and 20 ng/ml was 10.9% and 27.6%, respectively. 25(OH)D deficiency (table 1) was more prevalent with female gender, Asian background and medical co-morbidities (diabetes, hypertension, cardiovascular disease, obesity). At baseline there were clinically significant differences between the final study cohort and individuals excluded from the analysis; in the prevalence of diabetes (10.8 vs. 6.7%), CVD (10.3 vs. 6.9%) and UACR (21.2 vs. 13.2 mg/g), (supplementary table 2).

Incident Albuminuria

Of the 5849 participants without albuminuria at baseline, 222 (3.8%) developed albuminuria over the 5-year follow-up period. The overall annual age-adjusted incidence of albuminuria (table 2) was 0.75% (95% CI, 0.65-0.85). The incidence was higher in those with low 25(OH)D levels 1.50% (95% CI, 1.06-1.95) compared to those with high 25(OH)D levels 0.66 (95% CI, 0.56-0.76). In those with 25(OH)D levels <15 ng/ml, there was no significant difference between males and females.
On regression analysis (table 3) vitamin D deficiency was significantly associated with albuminuria in the unadjusted model (OR 1.84, 95% CI 1.29-2.62, p=0.001), and in the multivariate model (OR 1.71, 95% CI 1.12-2.61, p=0.01).

Further analysis was undertaken using 20 ng/ml to define 25(OH)D deficiency (supplementary table 1). A similar trend was observed overall, although the magnitude of the association was reduced, and was not significant in the full multivariate model (OR 1.32, 95% CI 0.94-2.07, p=0.1).

Given these positive associations, the relationship between vitamin D and albuminuria was further explored by dividing the study population into quartiles based on serum vitamin D levels (table 4). There was an increasing risk of albuminuria across the quartiles of decreasing 25(OH)D level (*p* trend = 0.02). A similar association was noted when the study population was divided into common clinical cut-off points (table 4). Participants with 25(OH)D levels of <15ng/ml had an increased likelihood of albuminuria compared to those with levels ≥ 30 in the fully adjusted model OR 1.68 (95% CI, 1.00-2.83, *p* = 0.05). Additional analyses using clinical cut-points of (< 10, 10-20, 20-30 and ≥ 30 ng/ml as well as < 20, 20-30 and ≥ 30 ng/ml) demonstrated similar results (supplementary table 3). Finally vitamin D was modeled as a continuous predictor (figure 2). The fully adjusted model demonstrates increasing probability (odds) of incident albuminuria with serum 25(OH)D levels <15 ng/ml. No interactions were found on the albuminuria and vitamin D deficiency relationship by gender, age, ethnicity and diabetes mellitus (all interaction *p*> 0.05).

Incident impaired eGFR

Of the 6034 participants without an impaired eGFR at baseline, 161 (2.7%) developed an impaired eGFR over the 5-year follow-up period; of those the proportion with stage 3 CKD was 88.7%. The overall annual age adjusted incidence of an impaired eGFR (table 2) was 0.58% (95% CI, 0.49-0.66). The age and sex standardized incidence was higher in participants with vitamin D deficiency 0.92% (95% CI, 0.56-1.30) compared to those without deficiency 0.59% (95% CI, 0.51-0.68).

Table 3 shows the result of the logistic regression models assessing the relationship of vitamin D deficiency on the 5-year incidence of an impaired eGFR. Vitamin D deficiency was not associated with incident eGFR in the unadjusted model OR 1.27 (95% CI, 0.81-2.01, p=0.3), or multivariate model OR 0.93, (95% CI, 0.56-1.66, p=0.8). No interactions were found on the albuminuria and impaired eGFR relationship by gender, age, ethnicity and diabetes mellitus (all interaction *P* >0.05).

DISCUSSION:

Our study examines the association between low serum 25(OH)D levels and the incidence of albuminuria and impaired eGFR in a large, population-based cohort of adults, with no prevalent CKD at baseline. In this cohort, the annual age-adjusted incidence of albuminuria and impaired eGFR was significantly higher in those individuals with a serum 25(OH)D <15ng/ml. We also demonstrated an association between vitamin D deficiency and the 5-year incidence of albuminuria, which remained significant after multivariate adjustment. Our study however did not demonstrate a significant association between vitamin D deficiency and the 5-year incidence of an impaired eGFR.

The cross-sectional association between albuminuria and vitamin D deficiency has been described in general population [15] and CKD cohorts[17]. Our study demonstrated the association between vitamin D deficiency and the 5-year incidence of albuminuria, further emphasizing the potential role of vitamin D deficiency in the pathogenesis of albuminuria. Small, but well designed, short-term interventional trials have shown that the administration of vitamin D compounds leads to a transient reduction of proteinuria [30-32], further emphasizing this association. Vitamin D may also attenuate proteinuria indirectly, through beneficial effects on blood pressure and insulin resistance[33].

In contrast to many previously published studies, our study did not demonstrate an association between vitamin D deficiency and incident impairment in the eGFR. The

association between vitamin D deficiency and progression to end stage kidney disease (ESKD) was explored in NHANES III, a general population based survey of 13328 US adults[16]. Individuals with a 25(OH)D level of <15 ng/ml had a higher incidence of ESKD (OR 2.64, 95% CI 1.00 – 7.05), with a median follow-up of 9.1 years. It is important to note that this population included a high proportion of participants with prevalent CKD, therefore at greater risk for CKD progression. In a smaller study of 168 predominantly Caucasian individuals with CKD stages 2-5, the relationship between vitamin D deficiency and CKD progression and mortality was examined[18]. With a mean follow up of 48 months, 25(OH)D level of <15 ng/ml predicted both time to death and ESKD on crude analysis and were also an independent predictor of the study outcomes after multivariate adjustment. In a larger cohort from the Cardiovascular Health Study, 25(OH)D levels of <15ng/ml were significantly associated with GFR loss over 4-years of follow up[34]. Conversely the was no association between 25(OH)D levels and incident albuminuria or impaired eGFR in a cohort from the Framingham Offspring Study cohort followed for a median of 7.8-years[35].

It is possible that our study and the previously published studies represent cohorts at different stages of CKD and risk of progression. It is likely that vitamin D deficiency contributes to existing renal injury, given the associations of low 25(OH)D with albuminuria and systemic inflammation[17] – key predictors of CKD progression. Therefore low 25(OH)D levels may be a more useful biomarker in those already at increased risk of renal impairment or with evidence of established renal damage. This may account for the stronger association between low serum 25(OH)D levels

and greater decline in eGFR observed in diabetics[34]. In most studies the association between serum 25(OH)D levels and clinical outcomes is strongest at the severe spectrum of deficiency, and this was the case in our cohort. However the exact level varies with different cohorts and clinical end-points. This may be further confounded by the complex interaction of ethnic and genetic differences in vitamin D metabolism[36]. Given the generally slow development of renal impairment, and failure of patients with early CKD to progress[37], longer follow-up may be needed to better delineate this association.

Numerous mechanistic links between vitamin D and renal disease have been proposed. Activation of the Wnt/β-catenin signaling pathway induces podocyte injury in animal models[38], and this can be blocked by paricalcitol administration[39]. Many of the reno-protective effects of vitamin D are mediated by the RAS and NF-KB pathways[1]. Mice lacking the vitamin D receptor or the enzyme α-hydroxylase (required for 25(OH)D activation), constitutively over-express renin and develop hypertension[40], and the administration of vitamin D analogues decreases renin and angiotensin II expression[41]. Angiotensin II is a key mediator of renal damage and proteinuria through hemodynamic (vasoconstriction) and non-hemodynamic (cell proliferation, fibrosis and oxidative stress) means[42]. NF-KB is implicated in inflammation and fibrogenesis that is associated with CKD[43]. Fibroblasts derived from mice lacking the vitamin D receptor show intrinsic activation of NF-KB[12], and administration of paricalcitol to mice with experimental obstructive nephropathy blocked NF-KB and decreased interstitial inflammation[44]. Given theses strong mechanistic links it is not surprising that the administration of vitamin D analogues in

animal models of renal injury has been shown to attenuate fibrosis[44], glomerulosclerosis[45] and CKD progression[46].

Further insights into the interplay between vitamin D and CKD, can be gained by examining the physiological changes that occur in the kidney in response to kidney damage and vitamin D deficiency. In health, megalin mediated reuptake of filtered 25(OH)D is essential for renal autocrine calcitriol synthesis and to maintain adequate 25(OH)D levels in the circulation[47]. Vitamin D deficiency reduces the amount of 25(OH)D that is filtered, and available for reuptake, re-circulation and autocrine calcitriol synthesis by the proximal tubular cells[48]. Furthermore reduction in renal function is paralleled by a progressive decline in renal megalin expression[49]. It is therefore possible that the combination 25(OH)D deficiency and CKD act synergistically to further compound this problem.

The strengths of this study include the recruitment and prospective follow-up of a large, national, population-based cohort, a standardized interview and examination process and direct follow-up of all study participants at 5 years. All biochemical tests were performed in a central laboratory and in contrast to previous studies, use of the CKD-EPI equation is more likely to reflect clinically significant renal impairment. However there are also several limitations to consider. The observational nature of the study does not account for unmeasured confounders. The baseline 25(OH)D levels may not be a true reflection of lifetime levels, although recent data suggests that vitamin D status tends to remain stable over time[50]. Serum creatinine and urinary albumin were recorded as single measurements, introducing the potential

for misclassification bias and the 5-year follow-up period may be insufficient to detect decline in the eGFR. A large proportion of the original study cohort was excluded from the 5-yr study due to loss to follow-up and missing data. These individuals appeared to have more medical co-morbidities than those included in the study. It is possible that a greater prevalence of diabetes and CVD would increase the risk of developing albuminuria and increase the rate of decline of GFR, therefore potentially confounding the relationship between vitamin D and CKD described in this study. Calcitriol levels and other markers of mineral metabolism (such as parathyroid hormone or fibroblast growth factor-23) were not measured, and may confound the relationship between 25(OH)D levels and CKD progression. The use of calcitriol was not recorded, however this would be negligible given the low prevalence of CKD and Australian prescribing guidelines. Similarly the use of vitamin D supplements would be reflected in the serum 25(OH)D levels. The use of medications known to affect CKD progression, such as ACE-inhibitors and angiotensin receptor blockers, was not recorded. These may confer a protective effect over and above that of blood pressure control, and may therefore decrease any positive effect of vitamin D observed.

Many questions pertaining to vitamin D and CKD require further studies. The optimal 25(OH)D levels have not been established, and these may vary depending on the underlying disease state or the population studied. The negative results of early population based supplementation studies[51,52] have increased speculation that current guidelines for vitamin D replacement are too conservative. Current doses used for supplementation are generally low, and often unable to achieve a sustained

improvement in serum 25(OH)D levels, particularly in cases of severe deficiency[53]. The ideal way to replace vitamin D in CKD also remains contentious, given the widespread use of calcitriol to treat elevated PTH levels. Given the pleuripotent effects of vitamin D and new insights into its many actions, the exact role of "nutritional" and "active" vitamin D compounds, their dose, timing of intervention and desired target levels need to be re-evaluated.

In summary our study demonstrates a higher age-adjusted annual incidence of albuminuria and of an impaired eGFR in those who are vitamin D deficient. We have also shown that vitamin D deficiency independently predicts the 5-year incidence of albuminuria, but not the 5-year incidence of an impaired eGFR, in a general population cohort. Given this association, combined with the available experimental data it is tempting to speculate that the use of vitamin D compounds and correction of vitamin D deficiency may present a novel strategy to positively influence the development of CKD. These results need further evaluation in prospective, long-term, adequately powered clinical trials. These need to demonstrate that a sustained increase in 25(OH)D levels attenuates the development and progression of albuminuria and decline in renal function, and further establish the optimal target levels in the setting of CKD.

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Table 1: Characteristic of the cohort by serum 25(OH)D status (patients with baseline impaired eGFR and albuminuria excluded)

	Serum 25(OH) vitamin D level ng/ml			
Characteristic	**Total (n=5738)**	**≥ 15 (n=5115)**	**< 15 (n=623)**	**p**
Age (years)	50.6 [12.3]	50.6 [12.3]	51.2[11.8]	0.2
Age group				0.1
25-44	1881 [32.8]	1699 [33.2]	182 [27.4]	
45-64	3020 [52.6]	2669 [52.2]	351 [55.0]	
≥65	837 [14.6]	747 [14.6]	90 [17.5]	
Gender (female)	3139 [54.7]	2685 [52.5]	454 [72.9]	<0.001
Country of Birth				<0.001
AUSNZ	4371 [76.2]	3964 [77.5]	407 [65.3]	
English speaking	696 [12.1]	625 [12.2]	71 [11.4]	
Asia	233 [4.1]	149 [2.9]	84 [13.5]	
Season				<0.001
Summer (Dec-Feb)	657 [11.4]	630 [12.3]	27 [4.3]	
Autumn (Mar-May)	1355 [23.6]	1213 [23.7]	142 [22.8]	
Winter (Jun-Aug)	1902 [33.1]	1627 [31.8]	275 [44.1]	
Spring (Sep-Nov)	1824 [31.8]	1645 [[32.2]	179 [28.7]	
Smoker	623 [11.4]	579 [11.3]	73 [11.7]	0.7
Diabetes	337 [5.9]	283 [5.5]	54 [8.6]	0.002
Hypertension	21.6 [0.54]	21.2 [0.57]	25.2 [2.18]	0.02
SBP (mmHg)_	127.7 [16.8]	127.4 [16.7]	129.6 [17.6]	0.002
DBP (mmHg)	70.0 [11.5]	69.9 [11.4]	70.4 [11.5]	0.3
History of CVD	331 [5.8]	282[5.5]	49 [7.9]	0.02
BMI (kg/m^2)				<0.001
< 25	2233 [38.9]	2020 [39.5]	213 [34.2]	
25-30	2317 [40.4]	2087 [40.8]	230 [36.9]	
≥ 30	1188 [20.7]	1008 [19.7]	180 [28.9]	
Total cholesterol (mg/dl)	216.9 [39.8]	216.9 [39.1]	228.1 [42.2]	<0.001
UACR (mg/g)	6.08 [4.42]	5.98 [4.32]	6.87 [5.19]	<0.001
eGFR (ml/min per 1.73 m^2)	97.1 [14.0]	96.9 [14.0]	99.3 [14.1]	<0.001

Cell contents are either mean (SD) or number (%)
AUSNZ = Australia and New Zealand, BMI = body mass index, CVD = cardiovascular disease, SD = standard deviation
eGFR = estimated glomerular filtration rate, UACR = urine albumin to creatinine ratio, SBP = systolic blood pressure, DBP = diastolic blood pressure

Table 2: Age-standardized annual incidence of albuminuria and impaired eGFR

	Age- and sex-standardized incidence (%)			
Impaired eGFR	Male	Female	*P* value	Overall
25(OH)D <15 ng/mL	0.72 (0.18 – 1.29)	1.00 (0.55 – 1.48)	0.6	0.92 (0.56 – 1.30)
25(OH)D ≥15 ng/mL	0.51 (0.39 – 0.62)	0.69 (0.56 – 0.83)	0.4	0.59 (0.51 – 0.68)
Albuminuria	Male	Female	*P* value	Overall
25(OH)D <15 ng/mL	1.83 (0.97 – 2.74)	1.37 (0.86 – 1.88)	0.1	1.50 (1.06 – 1.95)
25(OH)D ≥15 ng/mL	0.79 (0.64 – 0.93)	0.53 (0.39 – 0.66)	<0.001	0.66 (0.56 – 0.76)

Data are incidence in percent per year (95% CI), age- and sex-standardized to the 1998 Australian population. eGFR (estimated glomerular filtration rate), 25(OH)D (25-hydroxyvitamin D). *P* value for the difference between males and females.

Table 3: eGFR <60 & Albuminuria and vitamin D regression models – 25(OH)D < 15ng/ml

	25-OH vitamin D < 15 ng/ml OR (95% CI)							
	Model 1[a]	***p***	**Model 2[b]**	**p**	**Model 3[c]**	***p***	**Model 4[d]**	***p***
eGFR < 60 (CKD-EPI)	1.27 (0.81-2.01)	0.3	1.16 (0.70-1.91)	0.56	1.03 (0.62-1.71)	0.9	0.93 (0.53-1.66)	0.8

	25-OH vitamin D < 15 ng/ml (OR (95% CI)							
	Model 1[a]	***p***	**Model 2[b]**	**p**	**Model 3[c]**		**Model 4[d]**	***p***
Albuminuria	1.84 (1.29-2.62)	0.001	2.10 (1.45-3.05)	<0.001	1.95 (1.34-2.86)	0.001	1.71 (1.12-2.61)	0.01

eGFR – estimated glomerular filtration rate, CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration,
OR – odds ratio, CI – confidence interval

[a] unadjusted
[b] adjusted for age and gender
[c] adjusted for age, gender, ethnicity, season, and latitude
[d] adjusted for age, gender, ethnicity, season, latitude, diabetes, body mass index, cholesterol, triglycerides, cardiovascular disease, smoking, baseline albumin: creatinine ratio, baseline estimated glomerular filtration rate and systolic blood pressure.

Table 4: Albuminuria Regression Models (Study population vitamin D quartiles and clinical cut-off points)

	Albuminuria OR (95% CI)			
25(OH)D level ng/ml	**Model 1[a]**	**Model 2[b]**	**Model 3[c]**	**Model 4[d]**
Vitamin D clinical cut-points				
≥30	Ref	Ref	Ref	Ref
15-29	1.18 (0.86-1.64)	1.15 (0.82-1.61)	1.09 (0.78-1.54)	0.97 (0.67-1.42)
<15	2.06 (1.36-3.15)[#]	2.33 (1.49-3.64)[+]	2.09 (1.32-3.33)[#]	1.68 (1.00-2.83)[*]
***P* trend**	0.002	0.001	0.007	0.1
	Model 1[a]	**Model 2[b]**	**Model 3[c]**	**Model 4[d]**
Study population vitamin D level (quartiles)				
≥31.3	Ref	Ref	Ref	Ref
24.8-31.3	0.95 (0.61-1.46)	0.90 (0.58-1.40)	0.87 (0.56-1.36)	0.83 (0.49-1.22)
19.2-24.8	1.58 (1.06-2.36)[*]	1.58 (1.05-2.39)[*]	1.51 (0.99-2.30)	1.47 (0.88-2.09)
<19.2	1.88 (1.27-2.77)[#]	1.94 (1.30-2.93)[#]	1.80 (1.18-2.74)[#]	1.47 (0.92-2.23)
***P* trend**	<0.001	<0.001	<0.001	0.02

OR - odds ratio, CI – confidence interval, 25(OH)D – 25-hydroxyvitamin D

* <0.05, [#] <0.01, [+]<0.001

[a] unadjusted

[b] adjusted for age and gender

[c] adjusted for age, gender, ethnicity, season, and latitude

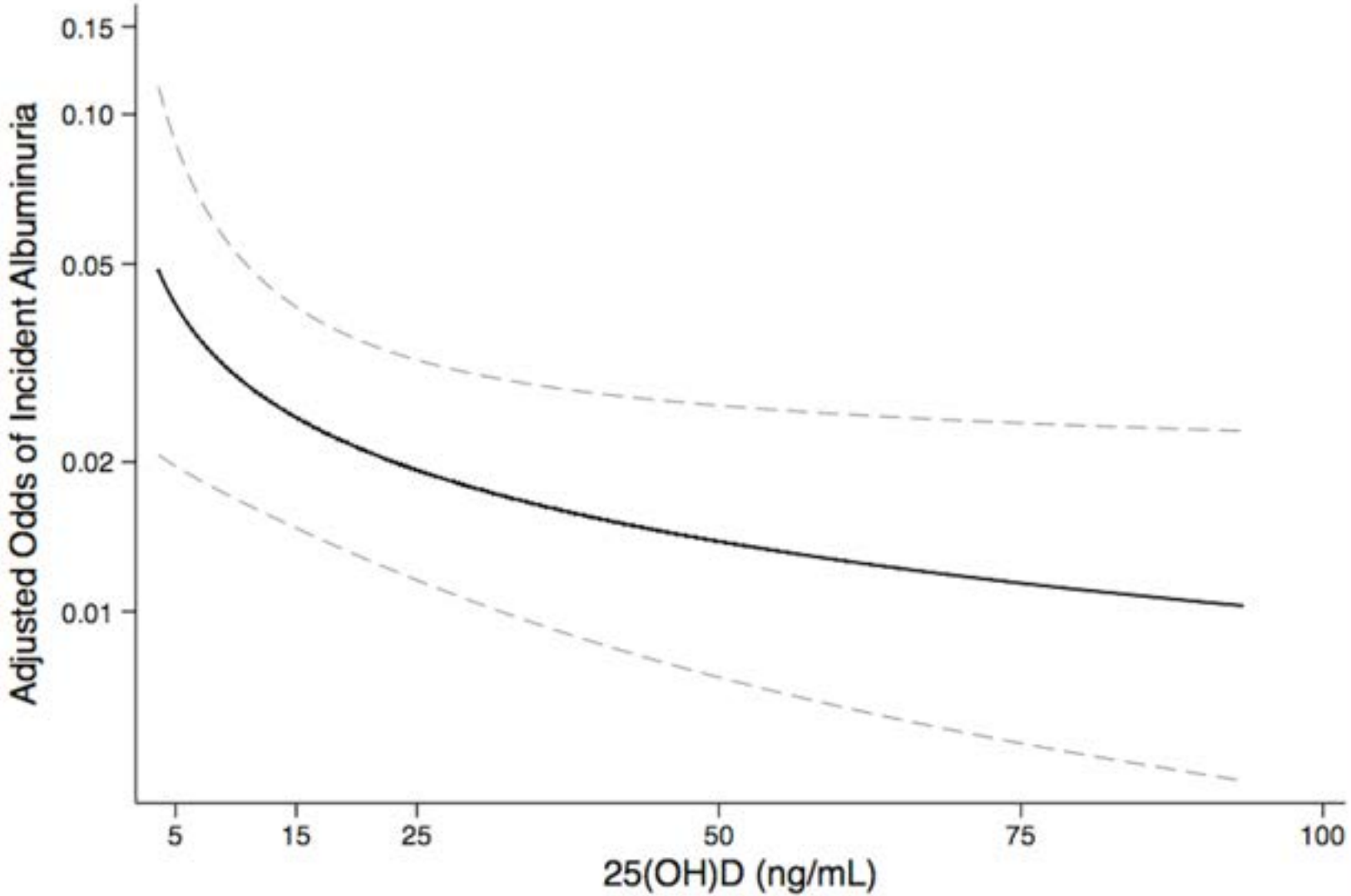
[d] adjusted for age, gender, ethnicity, season, latitude, diabetes, body mass index, cholesterol, triglycerides, cardiovascular disease, smoking, baseline estimated glomerular filtration rate, baseline albumin: creatinine ratio and systolic blood pressure.

Figure 1: Population flow during the study period and determination of final cohort

eGFR – estimated glomerular filtration rate

Figure 2: Adjusted odds of incident albuminuria at 5 years by baseline 25-hydroxyvitamin D [25(OH)D] concentration.

Curve represent odds for a subject who is a 51 year old white, female nonsmoker with diabetes mellitus, free of cardiovascular disease, systolic blood pressure 128 mmHg, cholesterol of 216 mg/dl, triglyceride 1.49mmol/L, eGFR 96 ml/min and uACR 6.1 mg/g. Sample taken in winter at latitude 31.7°S. Interrupted lines represents 95% confidence interval.


Adjusted Odds of Incident Albuminuria
0.15
0.10
0.05
0.02
0.01
5
15
25
50
75
100
25(OH)D (ng/mL)

Supplementary table 1: eGFR <60 & Albuminuria and vitamin D regression models – 25(OH)D < 20 ng/ml

	25-OH vitamin D < 20 ng/ml OR (95% CI)							
	Model 1[a]	***p***	**Model 2[b]**	**p**	**Model 3[c]**	***p***	**Model 4[d]**	***p***
eGFR < 60 (CKD-EPI)	1.35 (0.81-2.01)	0.08	1.14 (0.79-1.64)	0.4	1.02 (0.70-1.47)	0.9	1.12 (0.74-1.68)	0.6

	25-OH vitamin D < 20 ng/ml OR (95% CI)							
	Model 1[a]	***p***	**Model 2[b]**	**p**	**Model 3[c]**		**Model 4[d]**	***p***
Albuminuria	1.56 (1.18-2.06)	0.002	1.63 (1.22-2.18	0.001	1.53 (1.13-2.07)	0.005	1.32 (0.94-2.07)	0.1

eGFR – estimated glomerular filtration rate, CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration,
OR – odds ratio, CI – confidence interval

[a] unadjusted
[b] adjusted for age and gender
[c] adjusted for age, gender, ethnicity, season, and latitude
[d] adjusted for age, gender, ethnicity, season, latitude, diabetes, body mass index, cholesterol, triglycerides, cardiovascular disease, smoking, baseline albumin: creatinine ratio, baseline estimated glomerular filtration rate and systolic blood pressure.

Supplementary table 2: Comparison of final study cohort with individuals who were excluded

Characteristic	Final Study Population (n=6180)	Excluded Population (n=5067)	p
Age (years)	51.4	51.6	0.4
Gender female (%)	54.3	56.1	0.07
25(OH)D level (ng/ml)	25.8	25.2	<0.001
eGFR (ml/min per 1.73 m^2)	95.9	95.6	<0.3
UACR (mg/g)	13.2	21.2	<0.001
Diabetes (%)	6.7	10.8	<0.001
CVD (%)	6.9	10.3	<0.001
Smoker	11.3	21.4	<0.001
Chol (mg/dl)	220.4	220.4	0.2
BMI (kg/m^2)	26.9	27.1	0.003
SBP (mmHg)	128.8	130.2	<0.001

eGFR – estimated glomerular filtration rate, UACR = baseline albumin: creatinine ratio, CVD = cardiovascular disease. BMI = body mass index, SBP = systolic blood pressure

Supplementary table 3: Albuminuria Regression Models (Common vitamin D clinical cut-off points)

	Albuminuria OR (95% CI)			
25(OH)D level ng/ml	**Model 1[a]**	**Model 2[b]**	**Model 3[c]**	**Model 4[d]**
Vitamin D clinical cut-points				
≥30	Ref	Ref	Ref	Ref
20-30	1.11 (0.78-1.56)	1.07 (0.75-1.53)	1.02 (0.71-1.47)	0.94 (0.64-1.39)
10-20	1.57 (1.10-2.26)[+]	1.59 (1.09-2.33)*	1.47 (0.99-2.18)	1.19 (0.77-1.84)
<10	2.47 (1.26-4.84)[#]	3.10 (1.53-6.27)[#]	2.67 (1.29-5.55)[#]	2.13 (0.95-4.75)
***P* trend**	0.002	0.001	0.006	0.1

	Albuminuria OR (95% CI)			
25(OH)D level ng/ml	**Model 1[a]**	**Model 2[b]**	**Model 3[c]**	**Model 4[d]**
Vitamin D clinical cut-points				
≥30	Ref	Ref	Ref	Ref
20-30	1.11 (0.78-1.56)	1.07 (0.75-1.53)	1.02 (0.71-1.46)	0.94 (0.64-1.38)
<20	1.65 (1.16-2.35)[#]	1.70(1.18-2.46)[#]	1.55 (1.06 – 2.28)[#]	1.26 (0.82-1.92)
***P* trend**	0.004	0.004	0.02	0.2

OR - odds ratio, CI – confidence interval, 25(OH)D – 25-hydroxyvitamin D

* <0.05, [#] <0.01, [+]<0.001

[a] unadjusted

[b] adjusted for age and gender

[c] adjusted for age, gender, ethnicity, season, and latitude

[d] adjusted for age, gender, ethnicity, season, latitude, diabetes, body mass index, cholesterol, triglycerides, cardiovascular disease, smoking, estimated glomerular filtration rate, albumin: creatinine ratio and systolic blood pressure.