Negative effect of vitamin D on kidney function: a Mendelian randomization study

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ABSTRACT

Background. The kidney plays a central role in the regulation of vitamin D metabolism. It is not clear, however, whether vitamin D influences kidney function. Previous studies have reported conflicting results, which may have been influenced by reverse causation and residual confounding. We conducted a Mendelian randomization (MR) study to obtain unconfounded estimates of the association between genetically instrumented vitamin D metabolites and estimated glomerular filtration rate (eGFR) and the urinary albumin:creatinine ratio (UACR).

Methods. We performed a two-sample MR study based on three single nucleotide variants associated with 25(OH)D levels: rs2282679, rs10741657 and rs12785878, related to the genes GC, CYP2R1 and DHCR7, respectively. Estimates of the allele-dependent effects on serum 25(OH)D and eGFR/UACR were obtained from summary statistics of published genome-wide association meta-analyses. Additionally, we performed a one-sample MR analysis for both 25(OH)D and 1,25(OH)2D using individual-level data from six cohorts.

Results. The combined MR estimate supported a negative causal effect of log transformed 25(OH)D on log transformed eGFR ($\beta = -0.013, P = 0.003$). The analysis of individual-level data confirmed the main findings and also revealed a significant association of 1,25(OH)2D with eGFR ($\beta = -0.094, P = 0.008$). These results show that a 10% increase in serum 25(OH)D levels causes a 0.3% decrease in eGFR. There was no effect of 25(OH)D on UACR ($\beta = 0.032, P = 0.265$).

Conclusion. Our study suggests that circulating vitamin D metabolite levels are negatively associated with eGFR. Further studies are needed to elucidate the underlying mechanisms.

Keywords: albuminuria, causality, glomerular filtration rate, Mendelian randomization, vitamin D
INTRODUCTION

The kidneys play a central role in the regulation of vitamin D metabolism. They produce the 25-hydroxyvitamin D3-1α-hydroxylase enzyme that converts 25-hydroxyvitamin D3 [25(OH)D3] to the active form 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3]. As a consequence, during progression of chronic kidney disease (CKD), circulating levels of 1,25(OH)2D decline [1]. Conversely, whether vitamin D status might affect kidney function, is less clear. Because of the aforementioned interrelation between renal function and vitamin D metabolism, cross-sectional designs are not appropriate to address this question because they do not allow for reconstructing a temporal relationship between changes of vitamin D status and subsequent kidney function alterations. To date, several observational studies have investigated the effects of vitamin D status on renal function, with inconsistent results, with some suggesting a beneficial effect [2, 3], others reporting no association or mixed results [4–6] and others suggesting a harmful effect [7]. In all these studies, decline of kidney function was assessed from baseline to follow-up, whereas vitamin D status was measured at baseline only. As no information on vitamin D metabolites was available over time, the possibility that the findings might reflect reverse causation cannot be ruled out. Furthermore, due to the observational nature of these studies, the associations between vitamin D and renal function might be influenced by confounding, e.g. from inflammation, diabetes mellitus, hypertension or body mass index [8]. The Mendelian randomization (MR) approach analyses the relationship between exposure and outcome by an unconfounded genetic instrument. This approach might therefore be useful to overcome the limitations of observational studies [9, 10] and thus help to disentangle the relationship between vitamin 25(OH)D and markers of kidney function. In the present study, we investigated the relationship between the levels of circulating 25(OH)D and two measures of kidney function, namely the estimated glomerular filtration rate (eGFR) and the urinary albumin:creatinine ratio (UACR), with an MR approach using data from published literature. To further explore the association, we additionally analysed individual-level data from six population-based studies and extended the MR analyses to assess a causal effect of 1,25(OH)2D levels on eGFR. Because no genome-wide association study (GWAS) on 1,25(OH)2D levels was available, we generated and analysed individual-level data to close this gap and carry out an MR analysis for 1,25(OH)2D.

MATERIALS AND METHODS

Phenotypes and instrumental variables

In the initial two-sample MR, we included published genetic association summary statistics data on 25(OH)D [11], serum creatinine-based eGFR [12] and UACR [13] based on 33 868, 133 720 and 54 448 individuals, respectively. The one-sample, two-stage least-squares MR of 25(OH)D and vitamin 1,25(OH)2D on eGFR was calculated using data of 16 442 subjects from six studies (COLAUS, LURIC, ORCADES, PREVEND, SHIP and SHIP-Trend) and 5123 subjects from two studies (LURIC and PREVEND), respectively. None of the studies was included in the published 25(OH)D GWAS meta-analysis (Table 1, Supplementary Material). An additional 2696 samples of the PREVEND study without available genetic information but with measured levels of 25(OH)D and vitamin 1,25(OH)2D were included in the non-instrumented stratified analyses. All individuals analysed in this project were of European ancestry.

As an initial selection of the instruments, we considered single-nucleotide polymorphisms (SNPs) strongly associated with 25(OH)D levels as reported by Wang et al. [11] and Ahn et al. [15]. Given the different covariate adjustments and the smaller sample size of Ahn et al. compared with Wang et al., we focussed on the association results of the latter study only: the exonic SNPs rs4588 and rs2282679 in GC, rs12785878 in NADSYN1, rs10741657 upstream in CYP2R1 and rs6013897 downstream in CYP24A1. The β-values from the published GWAS meta-analyses on eGFR [12] and UACR [13] were used as effect estimates of the SNPs on kidney function. Since the SNP rs4588, which was also in strong linkage disequilibrium (LD) with rs2282679, was not included in these two meta-analyses, it was excluded from subsequent analyses.

All loci significantly associated with 25(OH)D in the study of Wang et al. [11] were evaluated as to whether they represented valid instruments for MR studies [16], taking into account both biological and statistical criteria. That is, SNPs were evaluated for a strong association with 25(OH)D and for potential pleiotropy [association with confounders of the association of vitamin D with kidney function to ensure that the SNPs were only associated with kidney function through 25(OH)D] [17]. Briefly, the SNP rs2282679 is located in an intron of GC that encodes a vitamin D–binding protein that transports vitamin D metabolites in the blood. The intronic SNP rs12785878 of NADSYN1 has several SNPs in high LD located in or near the 7-dehydrocholesterol reductase encoding gene DHCR7. This product of this gene as well as the cytochrome P450 family 2 subfamily R member 1 encoded by CYP2R1 is involved in vitamin D synthesis. However, we observed a significant association of rs6013897 with eGFR (P = 7.5 × 10−10). This SNP is located in close proximity to CYP24A1, which encodes an enzyme deactivating vitamin 1,25(OH)2D [18, 19] in the kidney as well as other tissues and thus we cannot exclude that the SNP affects kidney function via other mechanisms than exclusively through 25(OH)D levels. Therefore this SNP could not be considered as a valid instrument for the analysis of kidney function as an outcome and was excluded from the MR analyses. The F-statistics of the remaining three SNPs (rs2282679, rs10741657 and rs12785878) were higher than the recommended value of 10 [10] according to the ~6000 individuals included in Berry et al. [16], supporting these SNPs as strong instruments for both our two- and one-sample MR studies (Figures 1 and 2).

In Wang et al. [11], no effect estimates were available because the GWAS were pooled using a z-score-based meta-analysis, thus the effect estimates required for the MR as well as their standard errors were estimated using the provided allele frequency, effect direction, P-value and sample size as described previously [20]. The phenotypic variance was set to one, whereas the effect estimates represent the change of 1 standard
Table 1. Characteristics of cohorts included in the one-sample MR and non-instrumented analyses

<table>
<thead>
<tr>
<th>Study design</th>
<th>Genotyping platform</th>
<th>Vitamin D measurement</th>
<th>Serum creatinine measurement</th>
<th>Sample size</th>
<th>%</th>
<th>Age, years</th>
<th>BMI, kg/m²</th>
<th>Vitamin D, nmol/L</th>
<th>eGFR, ml/min/1.73 m²</th>
<th>Vitamin D, ng/mL</th>
<th>log[G(25OHD)]</th>
<th>SD</th>
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<tbody>
<tr>
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<td>LC-MS-MS and Jaffe kinetic compensated method</td>
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<td>89.9</td>
<td>48.5</td>
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<td>Jaffe-1997-2000</td>
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<td>10.4</td>
<td>10.6</td>
<td>19.7</td>
<td>9.8</td>
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<td>DiaSorin Liquid chromatography-tandem mass spectrometry</td>
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<td>14.5</td>
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The effect sizes of the kidney function traits correspond to a unit change of log transformed eGFR [four-parameter Modification of Diet in Renal Disease (MDRD) equation using calibrated creatinine measurements; log(eGFR)] and UACR values, respectively [12, 13].

The causal effect estimates of serum 25(OH)D on kidney function, based on the published GWAS meta-analysis results, were calculated using an inverse-variance meta-analysis of the two-sample MR triangulation per SNP [21], with its estimated effect sizes on 25(OH)D, and the effect sizes and their standard errors of the corresponding kidney trait as input.

MR based on individual study data of 25(OH)D and vitamin 1,25(OH)_{2}D on eGFR was calculated using a one-sample, two-stage least-squares analysis. The standardized residuals of the log transformed vitamin D traits adjusted for the same covariates as in the GWAS of Wang et al. [11] (i.e. sex, age, body mass index and season) were used as exposure [log(25(OH)D)] and log(1,25(OH)_{2}D), respectively. The log(eGFR) was used as outcome. In each study, the linearity of the relationship between log(25(OH)D) and log(eGFR) was assessed via locally weighted polynomial regression, as implemented in the R package lowess (R Project for Statistical Computing, Vienna, Austria). The SD of log(25(OH)D) that was needed to transform the effect estimates of the standardized residuals back to the original log scale for calculating the relative change of the causal effects was estimated in the SHIP cohort (σ = 0.44).

Simple (non-instrumented) association analyses were performed by fitting linear regression models of log(eGFR) values on log(25(OH)D) \((n = 19 \text{ } \text{ } 138)\) and log(1,25(OH)_{2}D) \((n = 7819)\), respectively, using the same cohorts and adjustments for covariates included in the one-sample MR (Table 1, Supplementary material). Stratified analyses were performed according to the CKD status, defined as eGFR < 60 ml/min/1.73 m².

All analyses were performed using the R software package [22]. Individual data analyses were performed in each cohort separately by distributing a centralized script and meta-analysing the results afterwards by inverse-variance weighting. The significance of the statistical tests was set at \(z = 0.025\), corresponding to a level of 0.05 divided by the two outcomes tested (eGFR and UACR).

RESULTS

The two-sample MR analysis for kidney function supported a negative causal effect of 25(OH)D levels on eGFR \(\beta = -0.013, P = 0.003; \text{ Figure 1, Supplementary data, Figure S1A}\), with

\[ \text{Supplementary data, Table S1} \]
relatively low heterogeneity between the three instruments ($I^2 = 28\%$). This result was reinforced by the one-sample MR analysis ($\beta = -0.033$, $P = 0.013$; Supplementary data, Figure S2A and Table S2). Additionally, a one-sample MR showed a significant association of vitamin 1,25(OH)$_2$D on eGFR ($\beta = -0.094$, $P = 0.008$; Supplementary data, Figure S2B and Table S2). No indication of a non-linear relation between these traits was observed (Supplementary data, Figures S4 and S5).

There was no evidence of a causal association between vitamin D and UACR ($\beta = 0.032$, $P = 0.265$, $I^2 = 0\%$; Figure 2 and Supplementary data, Figure S1B) using the association results of published GWASs.

FIGURE 1: Association results of MR analyses with eGFR. The figure shows the GWAS meta-analysis association results of the instruments (SNPs) with log[25(OH)D] and log[eGFR] (solid arrows) used to estimate the causal effect (dashed arrow) between 25(OH)D and eGFR through MR. a, b and c represent the association results of the respective instruments.

FIGURE 2: Association results of MR analyses with UACR. The figure shows the GWAS meta-analysis association results of the instruments (SNPs) with log[25(OH)D] and log(UACR) (solid arrows) used to estimate the causal effect (dashed arrow) between 25(OH)D and UACR through MR. a, b and c represent the association results of the respective instruments.

To investigate possible reasons for the previously reported conflicting association between lower levels of 25(OH)D and impaired kidney function [2, 5], we performed a non-instrumented association between 25(OH)D levels and eGFR stratified by CKD status. We observed a negative association between eGFR and 25(OH)D in the 18 029 non-CKD individuals ($\beta = -0.025$, $P = 1.1 \times 10^{-66}$; Supplementary data, Figure S3E) and a positive association in the 1109 CKD individuals ($\beta = 0.014$, $P = 3.1 \times 10^{-4}$; Supplementary data, Figure S3C). In contrast, vitamin 1,25(OH)$_2$D was positively associated with eGFR in both the 448 CKD subjects ($\beta = 0.044$, $P = 5.2 \times 10^{-13}$; Supplementary data, Figure S3D) and in the 7371 non-CKD individuals ($\beta = 0.006$, $P = 0.002$; Supplementary data, Figure S3F). Comparing the causal one-sample MR effects with the observed (non-instrumented) association results of eGFR using the same individuals, the effect directions were concordant for 25(OH)D ($\beta = -0.022$, $P = 7.2 \times 10^{-33}$; Supplementary data, Figure S3A) but not for vitamin 1,25(OH)$_2$D ($\beta = 0.037$, $P = 3.0 \times 10^{-33}$; Supplementary data, Figure S3B).

Based on the estimated causal effects, a 10% increase in serum 25(OH)D levels corresponds to a 0.3% decrease in eGFR (two-sample MR). The estimated change in 25(OH)D levels depending on the number of trait-increasing alleles per instrument varies from 3 to 18% (Figure 3), whereas individuals being homozygous for all trait-increasing alleles of these three SNPs are estimated to have 37% higher 25(OH)D levels compared with individuals without trait-increasing alleles.

DISCUSSION

Using the largest available GWAS on vitamin D metabolites and eGFR, we observed a potentially negative causal relationship between serum 25(OH)D and kidney function defined by
eGFR. This association could be reinforced by individual study MR analyses. Additionally, a similar negative causal association was revealed for vitamin D with eGFR.

Although these results are consistent with the observational association results of 25(OH)D and kidney function in the population-based cohorts participating in this study, they contradict the previously reported associations of lower vitamin D status with increased risk of CKD progression [2, 5] assessed as either rapid decline in eGFR or risk of incident end-stage renal disease. However, when limiting our observational analyses to individuals with CKD, the effect directions were concordant with those reported for CKD progression from previous observational studies. A potential explanation for this observation could be an impaired reabsorption of vitamin D metabolites in the proximal tubules due to substantially reduced kidney function, resulting in reduced serum vitamin D levels. This reverse causation could mask a possible causal effect of higher vitamin D levels on eGFR. The outcomes analysed in these two previous studies suggest that individuals with low kidney function at baseline are more likely to be included in the group of CKD progressors than the individuals with normal kidney function, which in turn could lead to confounding of the observed association of vitamin D status with kidney function. Supporting our explanation, other population-based longitudinal studies, which either excluded patients with CKD at baseline or did not use a rapid eGFR decline for case definition, did not show a significant positive association of vitamin D status with change in kidney function [3, 4, 6, 7].

Even though associations of vitamin D status with changes in albuminuria were reported previously [3, 6, 23], no causal effect was shown in our study using two-sample MR analysis. Besides the possibility that no true causal effect exists, our MR analyses included only cross-sectional measurements of UACR to assess albuminuria, it was limited in detecting non-linear log-log transformed causal associations of 25(OH)D on UACR and may still have been underpowered. Another issue might be the biological variability of UACR as well as the lower precision of UACR assessment in healthy individuals compared with eGFR because of limit-of-detection issues with the urinary albumin assay. The latter issue could affect the effect magnitude of genetic association estimates [24].

As both exposure and outcome were log transformed prior to the analysis, a non-linear causal relation between untransformed 25(OH)D and eGFR is likely. However, the causal effect with respect to the relative change on the original scale of the traits is rather small, suggesting that other factors not directly related to 25(OH)D levels are clinically more important for kidney function.

Several limitations apply to our study affecting the significant causal associations of vitamin D traits on eGFR. Because LURIC is a hospital-based cohort selected for an acute coronary syndrome, a one-time estimate of glomerular filtration rate may not accurately represent CKD status. However, the LURIC study was included in previous genetic analyses that gave rise to clear and homogeneous signals. The cross-sectional data limit possible interpretations of the molecular mechanisms underlying the causality. In particular, it would be of interest whether an increase in 25(OH)D levels causes a decline in eGFR or reduced 25(OH)D levels have a protective effect on kidney function.

There are different GFR estimating methods available, including the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which may provide more precise estimates in population-based samples compared with the MDRD formula [25]. However, no large-scale GWAS based on CKD-EPI eGFR was conducted until today. For this reason, we used the results of the GWAS of log transformed cystatin C–based eGFR, carried out on 33 152 participants by the CKDGen Consortium [12] to assess the robustness of our causal estimates. The causal effect of 25(OH)D on the cystatin-based eGFR was −0.014 (P = 0.11), thus almost identical to that obtained with the MDRD-based estimate. The lack of statistical significance is expected given the substantially smaller sample size.

Furthermore, there is no readily available explanation for our finding of a negative association between 25(OH)D and eGFR. Circulating levels of 25(OH)D are generally used to gauge vitamin D status, as they increase dose-dependently following vitamin D administration. However, the formation of 1,25(OH)2 D is tightly regulated and unlikely to increase due to higher levels of 25(OH)D, as shown by randomized controlled trials in which supplementation with vitamin D did not consistently cause an increase in 1,25(OH)2 D levels [26, 27]. That circulating levels of 25(OH)D might fail to capture the complexity of the relationship with kidney function is confirmed by the study of Rebholz et al. [5], in which the authors found no association between 25(OH)D and the risk of end-stage renal disease but did find a significant association for vitamin D–binding protein.

Vitamin D is a lipid-soluble hormone, whereas only free vitamin D is passing the cell membrane (with the exception of megalin-mediated vitamin D uptake in the proximal tubule of the kidney and the parathyroid gland) and interacts with the vitamin D receptor [28, 29]. Therefore, additional studies using measurements of free vitamin D could help to shed light on the biological mechanisms explaining the causal association revealed in our study.

Finally, all analyses were performed based on data of European ancestry individuals, thus the generalization of the findings with respect to other ethnicities needs to be evaluated.

In conclusion, our results demonstrate that levels of both 25(OH)D and 1,25(OH)2 D are negatively associated with eGFR. Further studies are warranted to confirm this finding and to look at potential mechanisms for this association.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online. An author video to accompany this article is available at: https://academic.oup.com/ndt/pages/author_videos.

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**AUTHORS’ CONTRIBUTIONS**

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**REFERENCES**


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