Impact of Vitamin D on Chronic Kidney Diseases in Non-Dialysis Patients: A Meta-Analysis of Randomized Controlled Trials

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Abstract

Background and Objectives: Recent studies have supported a role for both newer and more established vitamin D compounds in improving proteinuria, although systematic evaluation is lacking. Furthermore, concerns remain regarding the influence of vitamin D on the progression of renal function. We analyzed the efficacy and safety of vitamin D in non-dialysis patients and compared the use of newer versus established vitamin D compounds by performing a meta-analysis of randomized controlled trials.

Design: A literature search of PubMed (1975 to September, 2012), EMBASE.com (1966 to September, 2012) and Ovid EBM Reviews (through September, 2012) was conducted.

Results: Eighteen studies were eligible for final inclusion; of these, six explored the effects of vitamin D on proteinuria, twelve studied the effects of supplementation on renal function, and fifteen discussed the incidence of hypercalcemia. Compared to the placebo or no interference, both the newer and established vitamin D sterols reduced proteinuria to a similar extent (RR, 2.00; 95% CI, 1.42 to 2.81). No decrease in the glomerular filter rate was observed (SMD, −0.10; 95%CI, −0.24 to 0.03), and the risk for dialysis initiation was 1.48 (95% CI, 0.54 to 4.03) with vitamin D treatment. Additionally, there was an increased risk of hypercalcemia for patients treated with either newer or established vitamin D compounds as compared with the controls (RR, 4.78; 95% CI, 2.20 to 10.37). The head-to-head studies showed no differences in the effects of either newer or established compounds on proteinuria or the risk of hypercalcemia. No serious adverse events were associated with the administration of vitamin D.

Conclusions: Vitamin D therapy appears to decrease proteinuria and have no negative influence on renal function in non-dialysis patients. But the occurrence of hypercalcemia should be evaluated when vitamin D is provided. No superiority for newer versus established vitamin D analogue is found.

Introduction

End-stage renal disease (ESRD) imposes significant health and economic burdens on both individuals and communities [1]. Microalbuminuria is one of the earliest clinical manifestations of nephropathy and is associated with substantial risk for progressive kidney disease. Additionally, albuminuria predicts cardiovascular events, all-cause mortality and hospitalization for congestive heart failure [2]. Recent data have shown that increased proteinuria and decreased glomerular filtration rate (GFR) serve as independent predictors of all-cause mortality [3,4]. Thus, reducing proteinuria and protecting kidney function at the disease stages prior to dialysis are pivotal for preventing long-term kidney loss and other adverse events.

Renin-angiotensin system (RAS) inhibitors can reduce proteinuria and delay kidney dysfunction in patients with chronic kidney disease (CKD), but are unsuitable for those with advanced renal dysfunction due to the potential for renal deterioration and hyperkalemia. The exploration of other therapeutic modalities is urgently needed for CKD treatment. Although animal experiments have revealed that vitamin D can reduce proteinuria [5], the majority of existing clinical data have focused on the effect of vitamin D on mineral metabolism and bone diseases related to secondary hyperparathyroidism. Of the limited clinical studies that have explored extra-skeletal benefits of vitamin D, the VITAL trial (selective vitamin D receptor activation with paricalcitol for the reduction of albuminuria), a well-designed and relatively large-scale study, has shown promising but borderline significant results concerning albuminuria improvement [6]. In addition, it remains
unclear whether vitamin D treatment may harm renal function. Vitamin D therapy has been widely used in the management of CKD, traditionally in the form of ergocalciferol (vitamin D2), cholecalciferol (vitamin D3), calcitriol (1, 25 dihydroxyvitamin D3) and alfalcacidol (1α-hydroxyvitamin D3). However, the newer vitamin D analogues, including paricalcitol, doxercalciferol, 22-oxacalcitriol and falecalcitriol, play an increasingly important role in CKD treatment based on the experimental results of similar or better suppression of parathyroid hormone and possibly less calcemic effect compared with established vitamin D sterols [7]. While it is still uncertain whether newer compounds are superior to the established ones in terms of albuminuria improvement, renal function protection, hypercalcemia and other side effects reduction. The different forms of vitamin D compounds were listed in Table 1.

Given the fact that vitamin D is generally deficient and metabolically disordered in patients with CKD [8,9], supplementation of vitamin D may be significant throughout CKD evolution, especially at early and moderate stages. To our knowledge, few comprehensive meta-analyses and systematic reviews have explored the influence of vitamin D on proteinuria and the progression of CKD in non-dialysis patients or compared treatments between newer and more established sterols. In this regard, we performed a meta-analysis to clarify these issues, and we also evaluated hypercalcemia and other adverse events. The protocol of this analysis is available in File S1 and the search strategies are listed in File S2.

### Design and Methods

#### Study inclusion and exclusion criteria

Data from randomized controlled clinical trials (RCTs) that included patients receiving vitamin D in the study group and patients receiving placebo or no medications as controls were eligible for analysis. RCTs that compared newer and established vitamin D analogues were also included. Subjects who suffered from CKD should have no need for dialysis or renal transplantation at baseline. We considered the parameters of albuminuria, GFR, the risk of hypercalcemia and other adverse effects in these trials. The exclusion criteria consisted of incomplete relevant parameters required for our analysis, as unobtainable from the respective author and unable to be analyzed by statistical methods.

No restriction was set for language, publishing year or country to maximize the extent of the searches.

#### Data search strategies

We performed literature searches of PubMed (1975 to September, 2012), EMBASE.com (1966 to September, 2012) and OvidSP (through September, 2012) for the key words “vitamin d” or “vitamin d2” or “vitamin d3” or “calciferol” or “calcitriol” and “kidney disease” or “nephropathy” with the limitation of “controlled clinical trial”. Detailed data search strategies are given in File S2. Google Scholar was searched as a complementary measure for full-text articles. The EMBASE.com database is composed of Embase (from 1974) and majority of data from Medline (from 1966). OvidSP contains seven sub-databases including the Cochrane Library. Abstracts presented at meetings of the American Society of Nephrology, National Kidney Foundation, World Congress of Nephrology, American Diabetes Association, European Association for the Study of Diabetes and International Diabetes Federation in recent years were searched for additional studies. We used the Endnote X4 program for literature management and selection.

#### Data extracted

The following information was summarized by using a predefined data collection form: title, the first author’s name, country, mean age, year of publication, drug dosage, controls and causes of CKD. For binary outcomes, the number of cases and controls was recorded. For continuous data, the numbers, mean values and standard deviations of changes from baseline in the study group and the control group were recorded. If the 95% confidence interval was provided instead of the standard deviation, the standard deviation was calculated based on the equation provided in Cochrane Handbook. If the baseline and final standard deviations were given and the changes in the standard deviations were unknown, the correlation coefficient method advised by Follmann was used to calculate the values [10].

Two reviewers (Dr. L.J.X and Dr. X.S.W) screened the search results based on the inclusion and exclusion criteria. The two reviewers independently extracted useful data from the selected trials. When it was considered desirable and potentially useful, we contacted the investigators for additional information. Discrepan-

### Table 1. Vitamin D and derivatives.

| Vitamin D2 and derivatives | Vitamin D3 and derivatives |
|-----------------------------|---------------------------|
| **The established vitamin D compounds** | | |
| Parent compound | Vitamin D2 | Vitamin D3 |
| Ergocalciferol | Cholecalciferol |
| **Product of first hydroxylation** | | |
| 25-hydroxyvitamin D2 | 25-hydroxyvitamin D3 |
| **Product of second hydroxylation** | | |
| 1,25-Dihydroxyvitamin D2 | 1,25-Dihydroxyvitamin D3 |
| **The newer vitamin D analogues** | | |
| Full term | 1α,25-Dihydroxyvitamin D2 | F6-1α,25-Dihydroxyvitamin D3 |
| synonyym | doxercalciferol | Maxacalcitol |
| synonyym | Paricalcitol * | Falecalcitriol |

*In some literatures, paricalcitol is considered as the derivative of calcitriol.

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cies between the two reviewers were arbitrated by Professor YBL. Relevant missing data were sought by contacting the original author of the respective study.

The following parameters were accumulated: 1) albuminuria improvement (the numbers of patients who had a proteinuria reduction after treatment were recorded, according to the urine albumin/creatinine ratio or 24-hour urine protein excretion); 2) GFR changes (GFR was calculated according to the Modification of Diet in Renal Disease (MDRD) equation, the Cockcroft-Gault method, estimations of the continuous infusion of iothalamate, or predictions of the creatinine clearance rate (CCR) in the original trials, and differences in GFR changes were compared between the study and control groups); 3) incidence of hypercalcemia (hypercalcemia was defined as concentrations of serum calcium above 2.54–2.80 mmol/L (10.2–11.2 mg/dL); and 4) adverse events (all adverse events except for hypercalcemia were summarized in our analysis).

Study quality assessment
We used Revman 5.1 software (the Cochrane Collaboration, Copenhagen, Denmark) to evaluate the study quality. Two reviewers (Dr. LJX and Dr. FFZ) conducted these assessments independently, and disagreements were resolved through discussion between the two reviewers. The evaluation criteria consisted of the following: 1) random sequence generation; 2) allocation concealment; 3) blinding of participants and personnel; 4) blinding of outcome assessment; 5) incomplete outcome data; 6) selective reporting; and 7) other bias.

Statistical analysis
Standardized mean differences (SMD) and 95% confidence intervals (CI) were presented to compare the measurement data changes. SMD was used as a summary statistic in our analysis because the data for GFR conformed to the normal distribution; however, the measurement methods varied, and it was necessary to standardize the results to a uniform scale before they could be combined. Dichotomous data were expressed as risk ratios (RR) and 95% CI. In our analysis, the numbers of patients with proteinuria reduction, renal deterioration, hypercalcemia and other events were considered dichotomous data. Heterogeneity was analyzed using a $\chi^2$-squared test on n-1 degrees of freedom, with $\alpha=0.05$ used for statistical significance and $I^2$ for the degree of heterogeneity. Values of $I^2$ less than 25% indicated low heterogeneity, values near 50% indicated moderate heterogeneity, and those above 75% represented high heterogeneity. An $I^2$ value $>50\%$ was considered indicative of substantial heterogeneity. Subgroup analyses were then conducted based on year of the study, study participants, age of participants, design, interventions, and others, and careful consideration was given to the appropriateness of the meta-analysis. If the $I^2$ value was $>25\%$ or the results of an analysis clearly differed from those of other studies, a sensitivity analysis was conducted to assess the robustness of the outcomes.

Figure 1. Study flow diagram for the trials selection and exclusion.
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| Study          | Enrolled Country   | Sample size | Mean age (years) | Basal disease                                                                 | Renal function | Intervention Methods in study group | ACEI/ARB usage | Calcium usage | Study duration (months) | Outcomes in these trials |
|---------------|--------------------|-------------|-----------------|-------------------------------------------------------------------------------|----------------|-------------------------------------|----------------|----------------|------------------------|--------------------------|
| Nordal 1988 [11] | Norway             | 30          | 47.5            | nephritis, interstitial nephritis, DM, PKD                                    | 6–55 ml/min    | Calcitriol 0.25 µg daily, then 0.5 µg daily | not informed  | not informed | 8                      | hypercalcemia            |
| Hamdy 1995 [12]  | Belgium, France, Netherlands, UK | 176         | 52.0            | nephritis, HBP or DM                                                          | 15–50 ml/min   | Alfacalcidol 0.25 µg daily, adjusted to 1 µg daily | not informed  | When previously taken, continued | 24                      | Ccr, hypercalcemia       |
| Coburn 2004 [13] | the USA            | 55          | 64.6            | unclear                                                                      | 15–59 ml/min/1.73 m² | Doxercalciferol 1.0 µg/d, adjusted based on iPTH | not informed  | 16 patients with calcium in the two groups | 6                      | GFR                      |
| Rix 2004 [14]    | Denmark             | 36          | 52.5            | DM, nephritis, PKD, HBP                                                      | 10–60 ml/min   | Alfacalcidol 0.25–0.75 µg once daily  | not informed  | with no use of calcium | 18                      | Ccr, hypercalcemia       |
| Agarwal 2005 [15]| USA and Poland     | 195         | 62.2            | DM or other disease                                                           | 15–60 ml/min   | Paricalcitol initial dose of 1–4 µg/d | maintain concurrent therapies including ACEi/ARB | not informed  | 6                      | GFR                      |
| Coyne 2006 [16]  | the USA            | 220         | 62.7            | DM or other disease                                                           | 15–60 ml/min/1.73 m² | Paricalcitol 1 µg daily or 2 µg thrice weekly | not informed  | no use of calcium | 6                      | GFR, hypercalcemia       |
| Alborzi 2008 [17]| the USA            | 24          | 69.5            | DM, HBP, nephritis                                                           | GFR=30 ml/min  | Paricalcitol 1 µg or 2 µg daily     | a stable dose of an ACEi or ARB | not informed  | 1                      | GFR, proteinuria         |
| Fishbane 2009 [18]| the USA            | 55          | 57.8            | DM, HBP, nephritis, FSGS                                                      | 15–59 ml/min/1.73 m² | Paricalcitol 1 µg/d, adjusted based on iPTH | a stable dose of an ACEi or ARB | not informed  | 6                      | hypercalcemia proteinuria |
| Rucker 2009 [19]| Canada             | 128         | 69.0            | DM, HBP, nephritis, PKD, obstructive nephropathy                             | <30 ml/min/1.73 m² | Vitamin D3 1000 IU/d               | not informed  | 65 patients with calcium, comparable in the two groups | 3                      | GFR, hypercalcemia proteinuria |
| De Zeeuw 2010 [6]| Netherland, the USA, Denmark, Italy, Germany | 281        | 64.3            | DM                                                                           | 15–59 ml/min/1.73 m² | Paricalcitol 1 µg/day or 2 µg/day | Stable doses of ACEi or ARB | not informed  | 18 patients with calcium, comparable in the two groups | 6                      | hypercalcemia, proteinuria |
| Liu 2011 [20]    | China              | 50          | 35.9            | IgA nephropathy                                                              | >15 ml/min/1.73 m² | Calcitriol 0.5 µg twice weekly      | RASi at least 3 months | not informed  | 12                     | GFR, proteinuria         |
| Basturk 2011 [21]| Turkey             | 48          | 57.8            | DM or other disease                                                           | CKD stage 2–4 | Cholecalciferol 300,000 IU monthly | not informed  | not informed | 3                      | hypercalcemia            |
| Alvarez 2012 [22]| the USA            | 46          | 62.5            | DM, HBP                                                                      | CKD stage 2–4 | Cholecalciferol 50,000 IU/1–2weeks | not informed  | with no use of calcium | 13                     | hypercalcemia            |
| Kaerittichai 2012 [23]| Thailand       | 91          | 60.7            | DM                                                                           | >15 ml/min/1.73 m² | Calcitriol 0.25 µg twice weekly, then doubled | with no use of RASi | not informed  | 4                      | GFR, hypercalcemia proteinuria |
| Thadhani 2012 [24]| the USA, etc. Multi-national | 227        | 65.0            | HBP, DM or other disease                                                     | 15–60 ml/min/1.73 m² | Paricalcitol 2 µg/d, adjusted based on serum calcium | most patients with RASi | not informed  | 12                     | GFR, hypercalcemia proteinuria |
| Shroff 2012 [25]| UK                 | 47          | 9.3 (children)  | congenital abnormality, CKD stage 2–4 | Ergocalciferol | not informed | not informed | 13 children with calcium, comparable in the two groups | 12                     | GFR, hypercalcemia proteinuria |
Publication bias was assessed with funnel plots and Egger’s test. Both random-effect and fixed-effect models were used to pool the data, and the two models yielded mainly identical results in our analysis. The results were presented from the random-effect model. All statistical analyses were performed using Stata software (Stata version 11, College Station, Texas).

Results

We identified 769 full-text articles via database searches and 4 abstracts via manual internet searches through September 30th, 2012. Of these studies, 233 were from PubMed, 528 from Embase.com, and 8 from Ovid platform. After auto screening was performed using the Endnote program, 260 duplicate articles were removed, and 509 full-text articles were identified by manual screening. Of the 4 abstracts selected, 2 were excluded for lack of relevant data, and the remaining 2 were specific for trials that were reported in full-text articles. To obtain as much accurate information as possible, we contacted five corresponding authors regarding the incomplete or vague data available in their published works. Four of these authors kindly replied, but only one provided additional information that we needed. Three full-text articles were identified from the databases we listed above but downloaded from Google Scholar. Ultimately, 18 published studies [6,11–27] fulfilled our inclusion criteria. Figure 1 shows the study flow regarding trial selection and reasons for exclusion.

Trial characteristics

A total 1836 patients between the ages of 18~93 years, with CKD at stage 3~5, GFR values ranging from 6~60 ml/min/1.73 m² and no apparent need for dialysis or kidney transplantation at baseline, were included. The treatment duration ranged from 1 to 24 months (median: 6 months). Six of these trials explored the effect of vitamin D on proteinuria, twelve evaluated changes in renal function, and fifteen discussed the incidence of hypercalcemia in treated subjects as compared to controls. Two other studies compared the effects of newer versus more established vitamin D compounds in non-dialysis patients (Table 2).

Study quality

Most trials in our analysis were of moderate quality. Random sequence generation was clearly stated in 10 of 18 trials (56%). Allocation concealment was adequate in 5 of 18 trials (28%). Blinding of participants and personnel occurred in 12 of 18 trials (67%). Blinding of the outcome assessment was reported in 12 of 18 trials (67%). By contrast, outcome data were provided incompletely in 4 of 18 trials (22%), and selective reporting was found in 3 of 18 trials (17%). The likelihood of additional sources of bias was as high as 33% for 6 trials, and these related to declarations of interests or conflicts relating to the commercial source of the funding.

Outcome measurement

Proteinuria: Six RCTs (685 patients) compared the effects of vitamin D versus the use of placebo or no medication. Four of these studies evaluated a newer vitamin D analogue and the other two evaluated an established vitamin D compound. The pooled data indicated that vitamin D reduced proteinuria in non-dialysis patients (RR, 2.00; 95%CI, 1.42 to 2.81). The RR associated with the newer vitamin D sterol was 1.67 (95%CI, 1.22 to 2.29) and that for the established compound was 2.76 (95%CI, 1.60 to 4.74) (Figure 2). The subgroup analysis showed no difference between the newer vitamin D sterol and the established one (P=0.14).
also reviewed a study that compared the impact of the newer vitamin D analogue versus the established compound on proteinuria. To our regret, this original article did not provide concrete data, although it suggested that there was no difference between the newer compound and the established one [26].

GFR: Twelve RCTs (1124 patients) evaluated the effect of vitamin D therapy on GFR. After treatment, the changes in GFR were not different (−0.10, 95%CI: −0.24 to 0.03) between the study group and the control group. Advanced analysis indicated that neither established analogues such as calcitriol and alfacalcidol (−0.14, 95%CI: −0.32 to 0.03) nor newer analogues such as paricalcitol and doxercalciferol (−0.03, 95%CI: −0.33 to 0.26) led to deteriorations in renal function. The subgroup analysis showed no difference between the newer vitamin D sterol and the established one (P = 0.23). No head-to-head study was obtained from the database searches that compared the effect of newer vitamin D analogues versus established compounds on GFR in non-dialysis patients (Figure 3A).

Four RCTs (730 patients) listed the numbers of patients who progressed to terminal renal failure and required dialysis. One of these trials evaluated the established vitamin D sterol, and the other three evaluated the newer compound. Neither the established compound (RR, 3.00; 95%CI, 0.81 to 11.03) nor the newer compound (RR, 0.78; 95%CI, 0.32 to 1.89) was indicated to increase the risk of renal deterioration (pooled RR, 1.48; 95%CI, 0.54 to 4.03) (Figure 3B).

Incidence of hypercalcemia: Regarding the occurrence of hypercalcemia, thirteen RCTs (1378 patients) compared the newer vitamin D sterol or the established compound with placebo treatment or no medication, and two RCTs compared the newer compound with the established compound. The risk of hypercalcemia was clearly higher in patients given vitamin D therapy as compared with those given the placebo or no medication (RR, 4.78; 95%CI, 2.20 to 10.37). The RR associated with the newer vitamin D compounds was 6.16 (95%CI, 1.57 to 24.17), and that associated with the established compounds was 3.90 (95%CI, 1.43 to 10.66). No difference was discovered between the newer compounds and the established compounds based on the original head-to-head studies (pooled RR, 1.56; 95%CI, 0.27 to 9.17) (Figure 4).

Other events: A total of 9 RCTs, 1221 patients: The pooled results showed no differences regarding the risk of death (Figure 5), pre-mature withdrawal (Figure 6), adverse events (Figure 7A) or serious adverse events (Figure 7B) in patients given vitamin D therapy as compared to those given the placebo or no medication. No superiority was found for either treatment with the newer vitamin D compounds or the established compounds. The reasons for patient withdrawal included serious adverse events, such as

| Study ID | Events, Treatment (RR 95%CI) | Events, Control (RR 95%CI) | Weight |
|----------|-----------------------------|-----------------------------|--------|
| **Reduction of proteinuria** | | | |
| the newer compound | | | |
| Agarwal (2005) [15] | 2.08 (1.19, 3.62) | 29/94 | 15/101 | 20.51 |
| Alborzi (2008) [17] | 3.73 (0.57, 24.35) | 8/15 | 1/7 | 3.07 |
| Steven F (2009) [18] | 2.20 (1.08, 4.50) | 16/28 | 7/27 | 15.16 |
| Dick de Z (2010) [6] | 1.35 (1.01, 1.81) | 99/184 | 35/88 | 34.14 |
| Subtotal (I–squared = 20.4%, p = 0.288) | 1.67 (1.22, 2.29) | 152/321 | 58/223 | 72.88 |
| the established compound | | | |
| Liu (2011) [20] | 2.24 (1.13, 4.44) | 17/26 | 7/24 | 16.05 |
| Kairaettch (2012) [23] | 3.91 (1.61, 9.53) | 20/46 | 5/45 | 11.07 |
| Subtotal (I–squared = 0.0%, p = 0.319) | 2.76 (1.60, 4.74) | 37/72 | 12/69 | 27.12 |
| Overall (I–squared = 39.9%, p = 0.140) | 2.00 (1.42, 2.81) | 189/393 | 70/292 | 100.00 |
### Influence on glomerular filter rate

The newer compound

| Study ID | SMD (95% CI) | Treatment N, mean (SD) | Control N, mean (SD) |
|----------|--------------|------------------------|----------------------|
| Coburn (2004) [13] | -0.29 (-0.90, 0.32) | 22, -4.7 (7.25) | 20, -2.5 (7.97) |
| Agarwal (2005) [15] | 0.09 (-0.19, 0.38) | 94, -2.5 (5.24) | 101, -3 (5.33) |
| Coyne (2006) [16] | -0.21 (-0.51, 0.09) | 82, -2.52 (4.76) | 93, -1.57 (4.42) |
| Alborzi (2008) [17] | 1.20 (0.28, 2.11) | 16, 6.1 (10) | 8, 5.6 (9.27) |
| Thadhani (2012) [24] | -0.22 (-0.52, 0.07) | 88, -9.5 (25.3) | 91, -3.8 (25.8) |
| Subtotal (I^2 = 63.5%, p = 0.027) | -0.03 (-0.33, 0.26) | 302 | 313 |

The established compound

| Study ID | SMD (95% CI) | Treatment N, mean (SD) | Control N, mean (SD) |
|----------|--------------|------------------------|----------------------|
| Hamdy (1995) [12] | -0.13 (-0.47, 0.20) | 73, -5.7 (8.54) | 65, 4 (16.1) |
| Rix (2004) [14] | -0.44 (-1.16, 0.27) | 16, -28 (4) | 15, -26 (5) |
| Rucker (2009) [19] | -0.24 (-0.59, 0.11) | 65, -0.7 (3.4) | 63, 0.3 (4.9) |
| Liu (2011) [20] | -0.27 (-0.83, 0.29) | 26, -3.2 (12.2) | 24, 0.03 (11.6) |
| Krairittichai (2012) [23] | 0.00 (-0.41, 0.41) | 46, -1 (10.7) | 45, -1 (9.4) |
| Shroff (2012) * [25] | -0.11 (-0.73, 0.51) | 20, -2.1 (4.92) | 20, -1.6 (4.03) |
| Basturk (2011) [21] | 0.24 (-0.47, 0.94) | 16, 0.42 (12.2) | 15, 1.96 (7.19) |
| Subtotal (I^2 = 0.9%, p = 0.839) | -0.14 (-0.32, 0.03) | 262 | 247 |

Overall (I^2 = 21.4%, p = 0.234) -0.10 (-0.24, 0.03) 564 560

* Data were revised after we communicated with the author.

#### Risk of dialysis initiation

The newer compound

| Study ID | RR (95% CI) | Events, % | Weight |
|----------|-------------|-----------|--------|
| Coburn (2004) [13] | 0.96 (0.06, 14.50) | 1/25 | 1/24 | 11.84 |
| De Zeeuw (2010) [6] | 2.97 (0.36, 24.30) | 6/188 | 1/93 | 18.09 |
| Thadhani (2012) [24] | 6.00 (0.73, 49.03) | 6/112 | 1/112 | 18.11 |
| Subtotal (I^2 = 0.0%, p = 0.574) | 3.00 (0.81, 11.05) | 13/325 | 3/229 | 48.05 |

The established compound

| Study ID | RR (95% CI) | Events, % | Weight |
|----------|-------------|-----------|--------|
| Handy (1995) [12] | 0.78 (0.32, 1.89) | 8/89 | 10/87 | 51.95 |
| Subtotal (I^2 = 0.0%, p = 0.78) | 0.78 (0.32, 1.89) | 8/89 | 10/87 | 51.95 |

Overall (I^2 = 26.6%, p = 0.252) 1.48 (0.54, 4.03) 21/414 13/316 100.00

Figure 3. Effect of newer and established vitamin D compound on renal function versus controls respectively.
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progression to dialysis or cardiac events including congestive heart failure, myocardial infarction, atrial fibrillation, acute renal failure secondary to heart failure and pericardial effusion, pneumonia, stroke, and mortality, or loss of contact. Side effects that might have been unrelated to vitamin D treatment included gastrointestinal disturbances, pseudogout, upper respiratory tract infection, cough, constipation, urinary tract infection, paronychia, diarrhea, and others. In addition, two subjects had slightly raised hepatase levels and mild anaphylaxis potentially related to vitamin D therapy (Table 3).

Figure 4. Comparison of newer vitamin D sterol and established one versus controls, and comparison of newer vitamin D versus established one on the risk of hypercalcemia. doi:10.1371/journal.pone.0061387.g004

Heterogeneity and publication bias
Low to moderate heterogeneity was demonstrated in our analysis. The index $I^2$ value from RCTs analyzing proteinuria was 39.9% ($P = 0.14$), that related to GFR was 21.4% ($P = 0.23$), and that for hypercalcemia was very low (0.0%, $P = 0.74$). However, the index $I^2$ value from RCTs analyzing the risk for premature withdrawal was 52.9% ($P = 0.04$), and we explored potential reasons for this heterogeneity in the risk for premature withdrawals by subgroup analysis. We found that year of the study was a significant effect modifier and may have accounted for the heterogeneity in the premature withdrawal analysis (Table 4).
The sensitivity analysis of trials exploring proteinuria and premature withdrawal showed a high level of robustness, and trials evaluating GFR in relation to treatment with newer vitamin D compounds showed a low level of sensitivity. The funnel plots and sensitivity analysis results can be found in Figure S1 and Figure S2, S3, S4 for detail. Publication bias was not detected for studies concerning GFR and for those evaluating hypercalcemia (for Egger’s test, $P = 0.45$ and $0.80$, respectively; Figure S1). Studies that evaluated proteinuria, mortality, premature withdrawal, and adverse effects were inadequate for the assessment of publication bias.

**Discussion**

We performed a meta-analysis of available published studies to explore the effects of vitamin D therapy in non-dialysis patients and drew the conclusion that both newer vitamin D analogues and established compounds significantly reduced proteinuria in these patients. Although the clinical practice guidelines of KDIGO (Kidney Disease Improving Global Outcomes) have recommended vitamin D supplementation in patients with CKD mainly for treating mineral and bone disorders related to secondary hyperparathyroidism, recent clinical studies and experimental animal data have confirmed that the effects of vitamin D extend beyond mineral metabolism [6,28–33].

Tian et al. reviewed the benefits of vitamin D therapy, which include immunomodulatory and anti-inflammatory effects, vascular effects, regulation of the RAS and certain effects on glucose metabolism [28]. In animal studies, vitamin D monotherapy obtained an equivalent effect on proteinuria as compared to ARB and double benefits when combined with ARB [5]. Furthermore, in a large cohort evaluated for the Third National Health and Nutrition Examination Survey (NHANES III), a stepwise rise in the prevalence of albuminuria was reported with vitamin D insufficiency [29]. All the above suggested a potential intrinsic anti-proteinuric property of vitamin D.
With the development of dialysis techniques and kidney transplant operations for patients with ESRD, patient lifespan has been significantly extended, but quality of patient life has declined and costs have sharply increased. Controlling proteinuria at early stages and preserving residual renal function are no doubt significant; however, it should be noted that in 1978, a study published in the Lancet magazine reported that 18 subjects with advanced CKD demonstrated deteriorated renal function after vitamin D treatment [34]. But this conclusion was questioned due to the small sample size and short study duration, and the result was not supported by subsequent trials.

In our analysis, vitamin D therapy was not found to damage renal function, although it was also clear that vitamin D failed to improve GFR. This was surprising because decreases in albuminuria were not associated with kidney function improvement. One potential reason for this disparity may have been differences between trials in terms of study subjects. Furthermore, several risk factors (with the exception of proteinuria) are shown to correlate with the deterioration of renal function. Besides, studies have also
## A
### Risk of adverse events

#### the newer compound

| Study          | RR (95% CI) | Treatment | Control | %  |
|----------------|-------------|-----------|---------|----|
| Fishbane (2009) | 0.75 (0.33, 1.73) | 7/28      | 9/27    | 5.89 |
| Dick de Z (2010) | 0.96 (0.51, 1.80) | 26/188    | 12/83   | 9.87 |
| Thadhani (2012) | 1.02 (0.88, 1.19) | 70/88     | 71/91   | 74.18 |
| **Subtotal**    | 1.01 (0.87, 1.16) | 103/304   | 92/201  | 89.93 |

#### the established compound

| Study          | RR (95% CI) | Treatment | Control | %  |
|----------------|-------------|-----------|---------|----|
| Hamdy (1995)  | 7.82 (1.00, 61.22) | 8/89      | 1/87    | 1.01 |
| Liu (2011)    | 0.72 (0.32, 1.63) | 7/26      | 9/24    | 6.12 |
| Krairittichai (2012) | 0.68 (0.21, 2.25) | 4/44      | 6/45    | 2.94 |
| **Subtotal**  | 1.16 (0.35, 3.82) | 19/159    | 16/156  | 10.07 |

| Overall       | 0.98 (0.80, 1.21) | 122/463   | 108/357 | 100.00 |

## B
### Risk of serious adverse events

#### the newer compound

| Study          | RR (95% CI) | Treatment | Control | %  |
|----------------|-------------|-----------|---------|----|
| Coyne (2006)  | 1.18 (0.66, 2.09) | 20/101    | 18/107  | 32.88 |
| Fishbane (2009) | 0.96 (0.15, 6.37) | 2/28      | 2/27    | 3.06 |
| Dick de Z (2010) | 1.46 (0.79, 2.71) | 32/188    | 12/103  | 28.51 |
| Thadhani (2012) | 0.97 (0.55, 1.71) | 20/115    | 20/112  | 34.45 |
| **Subtotal**  | 1.17 (0.84, 1.62) | 74/432    | 52/349  | 98.91 |

#### the established compound

| Study          | RR (95% CI) | Treatment | Control | %  |
|----------------|-------------|-----------|---------|----|
| Liu (2011)    | 3.00 (0.13, 70.42) | 1/26      | 0/26    | 1.09 |
| **Subtotal**  | 3.00 (0.13, 70.42) | 1/26      | 0/26    | 1.09 |

| **Overall**    | 1.18 (0.85, 1.64) | 75/458    | 52/375  | 100.00 |

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Figure 7. Comparison of newer and established vitamin D sterols versus controls on adverse events and serious adverse events.
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indicated that renal impairments occur in the absence of albuminuria in some patients with diabetes, despite the classic histological features of diabetic nephropathy [35–37]. Although proteinuria improvement and renal function protection do not occur in parallel after vitamin D therapy in our analysis, series of studies [38–42] have invariably inferred that albuminuria reduction is important for future renal outcomes.

The development of hypercalcemia is a potential hazard related to vitamin D therapy. Although negative results were reported in specific RCTs, the pooled results indicated an increased probability of hypercalcemia after vitamin D therapy. This result is consistent with other meta-analyses that evaluated patients at all CKD stages [43], and these findings indicate that serum calcium concentrations should be clinically monitored when CKD patients are taking vitamin D supplements.

In this analysis, we obtained no evidence of superiority for either the newer vitamin D compounds or the established compounds in terms of their impact on proteinuria, renal function, hypercalcemia or other events.

To the best of our knowledge, this is the first meta-analysis to explore the reasons for heterogeneity in the trials that discussed the number of premature withdrawals.

Table 3. Adverse events mentioned in the trials.

| Study, year | Adverse events                                                                 | Conclusions                           |
|-------------|--------------------------------------------------------------------------------|---------------------------------------|
| Nordal, 1988 [11] | not informed                                                                    | not informed                          |
| Hamdy, 2005 [12] | gastrointestinal disturbances, pseudogout, renal failure, dialysis, default and death | not informed                          |
| Coburn, 2004 [13] | congestive heart failure, intestinal malabsorption, dialysis, myocardial infarction, presumed cardiac arrest, neuromuscular symptoms and other reasons | insignificant                          |
| Rix, 2004 [14] | not informed                                                                    | not informed                          |
| Agarwal, 2005 [15] | not informed                                                                    | not informed                          |
| Coyne, 2006 [16] | elevated liver enzyme levels, allergic reaction and death                       | insignificant                          |
| Alborzi, 2008 [17] | abdominal pain, acute renal failure                                             | insignificant                          |
| Fishbane, 2009 [18] | upper respiratory tract infection, cough, constipation, abdominal cramps, headache; congestive heart failure, episode of new atrial fibrillation and pneumonia | insignificant                          |
| Rucker, 2009 [19] | not informed                                                                    | not informed                          |
| De Zeeuw, 2010 [6] | diabetic gastroparesis, death, malaise, myalgia, pain, drug intolerance, erectile dysfunction, muscle spasms, edema | insignificant                          |
| Liu, 2011 [20] | upper respiratory tract infection, rash, urinary tract infection, paronychia, diarrhea, liver function disorder, hyperkalemia, joint pain, gout and renal calculus | renal calculus related to vitamin D |
| Basturk, 2011 [21] | not informed                                                                    | not informed                          |
| Alvarez, 2012 [22] | death                                                                          | insignificant                          |
| Krairittichai, 2012 [23] | upper respiratory tract infection, abnormal sweating , hyperglycemia, congestive heart failure | insignificant                          |
| Thadhani, 2012 [24] | worsening renal function and initiated long-term dialysis, other advise events | insignificant                          |
| Shroff, 2012 [25] | no ergocalciferol-related adverse events                                        | insignificant                          |
| Moe, 2011 [26] | quality of life indices on the SF-36 questionnaire measured between treatment groups | insignificant                          |
| Kovesdy, 2012 [27] | not informed                                                                    | not informed                          |

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Table 4. Subgroup analyses to explore the reasons for heterogeneity in the trials that discussed the number of premature withdrawals.

| Variable               | RR (95%CI); n Trials | P value |
|------------------------|-----------------------|---------|
| Number of participants | 0.56                  |         |
| ≥100                   | 1.60 (0.68 to 3.78); 4 |        |
| <100                   | 1.16 (0.61 to 2.22); 4 |        |
| Age of participants    | 0.13                  |         |
| ≤55 years              | 0.71 (0.40 to 1.26); 1 |        |
| 55–65 years            | 1.62 (0.85 to 3.11); 5 |        |
| ≥65 years              | 1.36 (0.79 to 2.34); 2 |        |
| Study duration         | 0.85                  |         |
| ≥12 months             | 1.26 (0.60 to 2.64); 3 |        |
| <12 months             | 1.40 (0.62 to 3.18); 5 |        |
| Type of medication     | 0.56                  |         |
| established vitamin D sterols | 1.07 (0.56 to 2.02); 3 | 0.56 |
| newer vitamin D sterols | 1.54 (0.71 to 3.31); 5 |       |
| Number of trial centers| 0.56                  |         |
| monocenter             | 1.18 (0.67 to 2.08); 5 |        |
| multicenter            | 1.72 (0.56 to 5.28); 3 |        |
| Year of the study      | 0.001                 |         |
| before 2005            | 0.67 (0.40 to 1.13); 2 |        |
| 2005–2009              | 1.11 (0.39 to 3.14); 2 |        |
| since 2010             | 2.57 (1.56 to 4.23); 4 |        |

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Vitamin D in Non-Dialysis Patients

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In summary, vitamin D therapy appears to decrease proteinuria and have no negative influence on renal function in non-dialysis patients. Thus, this treatment appears to be safe for CKD treatment, but the occurrence of hypercalcemia should be evaluated when vitamin D is provided. Furthermore, no superiority for newer versus established vitamin D analogues is found in non-dialysis patients, which implies that other factors such as expense or availability should be the first consideration for patients and practitioners.

Supporting Information

Figure S1 Funnel plots with pseudo 95% confidence limits to detect potential publication bias. The scatter plots represent individual studies for the indicated association. Egger’s test for publication bias was not significant in this analysis. (TIF)

Figure S2 Sensitivity analysis of trials exploring the amelioration of proteinuria with vitamin D therapy showed a low level of sensitivity, which indicates a robust result. (TIF)

Figure S3 Sensitivity analysis of trials evaluating GFR changes with newer vitamin D compounds therapy showed a low level of sensitivity. (TIF)

References

1. Collins AF, Foley RN, Chavers B, Gilbertson D, Herzog C, et al. (2012) United States Renal Data System 2011 Annual Data Report. Am J Kidney Dis 59 (1 Suppl 1): A7, e1–420.
2. De Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, et al. (2004) Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. Circulation 110: 921–927.
3. Knobler H, Zornitzki T, Vered S, Otinger M, Levy R, et al. (2004) Reduced glomerular filtration rate in asymptomatic diabetic patients: predictor of increased risk for cardiac events independent of albuminuria. J Am Coll Cardiol 44: 2142–2149.
4. Salles GF, Cardoso CR, Pereira VS, Fisman R, Mundfelit ES (2011) Prognostic significance of a reduced glomerular filtration rate and interaction with microalbuminuria in resistant hypertension: a cohort study. J Hypertens 29: 2014–2023.
5. Zhang Y, Deb DK, Kong J, Ning G, Wang Y, et al. (2009) Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: strong synergism with AT1 receptor antagonist. Am J Physiol Renal Physiol 297: F791–801.
6. De Zeeuw D, Agarwal R, Andalib M, Audhya P, Coyne D, et al. (2010) Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (NITAL study): a randomised controlled trial. Lancet 375: 1543–1551.
7. Slatopolsky E, Finch J, Brown A (2003) New vitamin D analogs. Kidney Int 63 (Suppl 85): S83–47.
8. Mohrota R, Kermah D, Bodoff M, Salusky IB, Mao SS, et al. (2008) Hypovitaminosis D in chronic kidney disease. Clin J Am Soc Nephrol 3: 1144–1151.
9. LaClair RE, Hellman RN, Karp SL, Kraus M, Ofer I, et al. (2005) Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States. Am J Kidney Dis 45: 1026–33.
10. Follmann D, Elliott P, Suh I, Caule J (1992) Variance imputation for purposes of clinical trials with continuous responses. J Clin Epidemiol 45: 769–773.
11. Nordal KP, Edel D (1985) Low Dose Calcitriol Versus Placebo in Patients with Predialysis Chronic Renal Failure. J Clin Endocrinol Metab 67: 929–936.
12. Hamdy NA, Kanis JA, Beneton MN, Brown CB, Juttmann JR, et al. (1995) Effect of alfalcaldiol on normal course orefinal bone disease in mild to moderate renal failure. BMJ 310: 358–363.
13. Coburn JW, Maun HM, Elango L, German MJ, Lindberg JS, et al. (2004) Doxercalciferol Safely Suppresses PTH Levels in Patients With Secondary Hyperparathyroidism Associated With Chronic Kidney Disease Stages 3 and 4. Am J Kidney Dis 43: 877–890.
14. Rix M, Eklind K, Olgaard K (2004) Effect of 18 months of treatment with alfalcaldiol on bone in patients with mild to moderate chronic renal failure. Nephrol Dial Transplant 19: 870–876.

Figure S4 Sensitivity analysis of trials inspecting premature withdrawal with vitamin D therapy showed a low levels of sensitivity. (TIF)

File S1 Study protocol for this meta-analysis. (DOC)

File S2 Database search strategies for this analysis. (DOC)

File S3 PRISMA checklist of this meta-analysis. (DOC)

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Author Contributions

Revised the draft and interpreted of data: ZMH GHW DHF WPD. Conceived and designed the experiments: LJX YBL XSW ZMH FFZ. Performed the experiments: LJX XSW ZMH FFZ. Analyzed the data: LJX FFZ. Contributed reagents/materials/analysis tools: LJX FFZ. Wrote the paper: LJX YBL.
29. De Boer IH, Ioannou GN, Kestenbaum B, Brunzell JD, Weiss NS (2007) 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). Am J Kidney Dis 50: 69–77.
30. Wang Y, Zhou J, Minto AW, Hack BK, Alexander JJ, et al. (2006) Altered vitamin D metabolism in type II diabetic mouse glomeruli may provide protection from diabetic nephropathy. Kidney Int 70: 882–891.
31. Deb DK, Chen Y, Zhang Z, Zhang Y, Szeto FL, et al. (2009) 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF-κB pathway. Am J Physiol Renal Physiol 296: F1212–F1218.
32. Zhang Z, Sun L, Wang Y, Ning G, Minto AW, et al. (2008) Renoprotective role of the vitamin D receptor in diabetic nephropathy. Kidney Int 73: 163–71.
33. Takano Y, Yamauchi K, Hiramatsu N, Kasai A, Hayakawa K, et al. (2007) Recovery and maintenance of nephrin expression in cultured podocytes and identification of HGF as a repressor of nephrin. Am J Physiol Renal Physiol 292: F1573–F1582.
34. Christiansen C, Rødbro P, Christensen MS, Hartnack B, Transbol I (1978) Deterioration of renal function during treatment of chronic renal failure with 1,25-dihydroxycholecalciferol. Lancet 2: 700–703.
35. Thomas MC, Machaa RN, Talasmandri C, Power D, Jerums G (2009) Nonalbuminuric renal impairment in type 2 diabetic patients and in the general population (national evaluation of the frequency of renal impairment co-existing with NIDDM [NEFRON] 11). Diabetes Care 32: 1497–1502.
36. Molitch ME, Stoffers M, Sun W, Rutledge B, Cleary P, et al. (2010) Development and progression of renal insufficiency with and without albuminuria in adults with type 1 diabetes in the diabetes control and complications trial and the epidemiology of diabetes interventions and complications study. Diabetes Care 33: 1536–1543.
37. Kramer CK, Lettko CB, Pinto LC, Silveira SP, Gross JL, et al. (2007) Clinical and laboratory profile of patients with type 2 diabetes with low glomerular filtration rate and normoalbuminuria. Diabetes Care 30: 1998–2000.
38. De Zeeuw D, Remuzzi G, Parving HH, Krane W, Zhang Z, et al. (2004) Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: Lessons from RENAAL. Kidney International 65: 2309–2320.
39. Remuzzi G, Chiurchiu C, Ruggenenti P (2004) Proteinuria predicting outcome in renal disease: nondiabetic nephropathies (REIN). Kidney Int Suppl 92: S90–96.
40. Ruggenenti P, Perna A, Gherardi G, Garini G, Zoccali C, et al. (1999) Renoprotective properties of ACE-inhibition in non-diabetic nephropathies with non-nephrotic proteinuria. Lancet 354: 359–364.
41. Remuzzi G, Chiurchiu C, Ruggenenti P (2004) Proteinuria predicting outcome in renal disease: nondiabetic nephropathies (REIN). Kidney Int Suppl 92: S90–96.
42. Hunsicker LG, Atkins RC, Lewis JB, Braden G, de Crespigny PJ, et al. (2004) Impact of irbesartan, blood pressure control, and proteinuria on renal outcomes in the Irbesartan Diabetic Nephropathy Trial. Kidney Int Suppl 66: S99–101.
43. Palmer SC, McGregor DO, Macaskill P, Craig JC, Elder GJ, et al. (2007) Meta-analysis: vitamin D compounds in chronic kidney disease. Ann Intern Med 147: B40–853.