Bone, Artery, & Renal Function in CKD

Supplementation of Vitamin D, Calcitriol, &/or Vitamin D Analogs

Disclosures: none

Objectives:

1. To assess the impact of vitamin D supplementation on bone, arterial, and renal function in patient with CKD (pre-dialysis)
2. To assess the impact of calcitriol supplementation
3. To assess the impact of vitamin D analog supplementation

Thomas A. Hughes, M.D.
Professor of Medicine - Retired
Division of Endocrinology, Metabolism, and Diabetes
University of Tennessee Health Science Center
HughesEndo.com

Vitamin D: Systemic & Paracrine Effects on Bone & Vessels

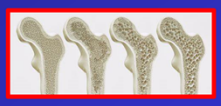
Nagpal et al: Endocrine Reviews 26:662-687, 2005

$1,25\text{-}(OH)_2D3$ binds to its receptor (VDR) dimerized to RXR **(3% of all genes)**

Co-activators: SRC, DRIP205, CBP/p300, TAFII

Most cells (not small intestine) have 1a-hydoxylase → not dependent on circulating calcitriol

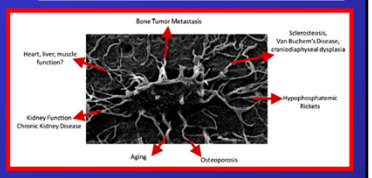
Some genes with Vitamin D response elements: (**Empty** VDR is a repressor on some genes)



Activates: (+ response element): *May be activated by 25-Vitamin D in MSCs

Bone Remodeling:	Osteocalcin*, RANKL (weak), TNAP*, NPP1 (+PPi)
Ca Binding:	**Trpv6**, Atp2b1, Calbindin-9k (S100g) (Intestinal Ca^{++} transport)
Metabolism:	Cyp3A4, Cyp3A1, Cyp3A11, 24-Hydroxylase → reduces calcitriol
Adhesion:	β-3 integrin (FGF-23 inhibitor?)
Anti-proliferation:	p21, IGFBP-3
Differentiation:	Involucrin, Phospholipase C gamma1

TNAP: Tissue Non-specific Alk Phos
NPP1: ATP → AMP + PPi



Inhibits: Probably the dominate Bone effect

NFAT, NF-kB (VDR competes with co-activators) →

Anti-inflammatory:	IL-2, IL-12, TNFa, IFNg, GM-CSF
Anti-proliferative:	EGF-R, c-myc, K16

Negative Response elements: PTH, PTHrP, Rel B (transcription activator)

NFAT: Nuclear Factor of activated T-Cells

S.M. Lee, J.W. Pike / Journal of Steroid Biochemistry & Molecular Biology 164 (2016) 265–270

The vitamin D receptor functions as a transcription regulator in the absence of 1,25-dihydroxyvitamin D3

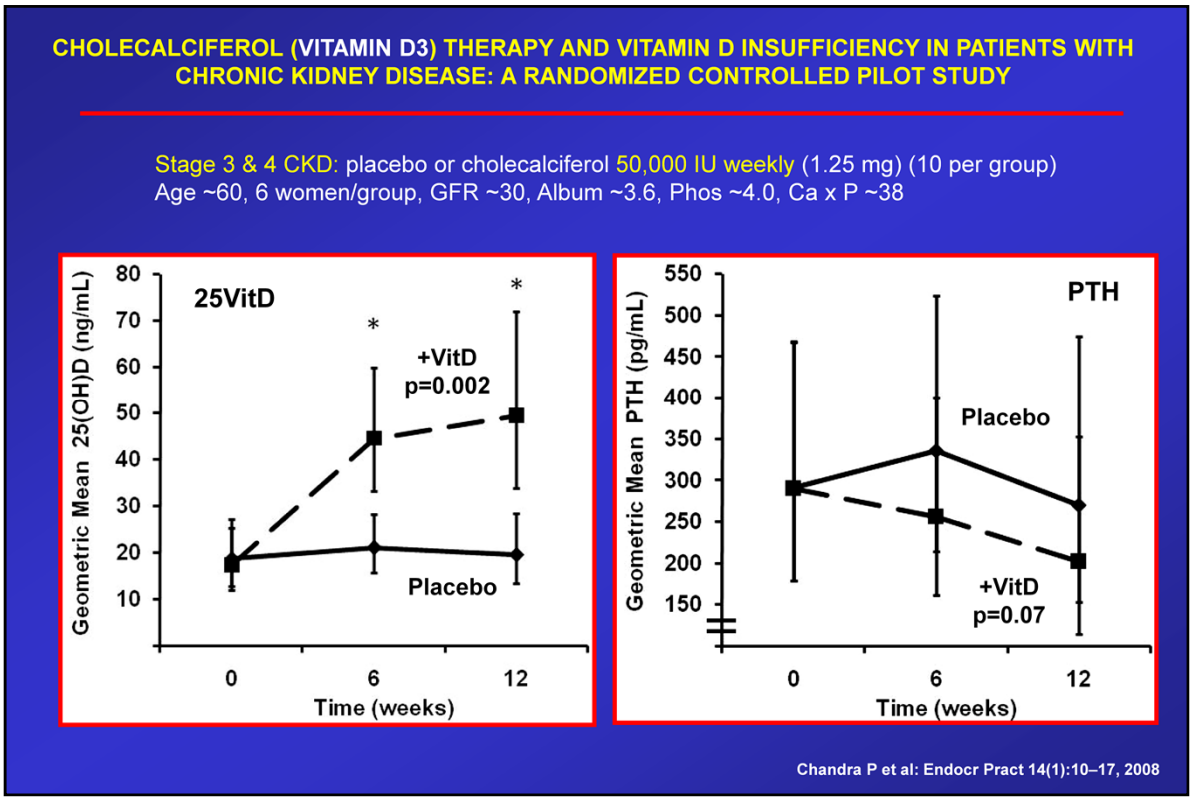
25-Hydroxyvitamin D3 induces osteogenic differentiation of human mesenchymal stem cells; Yan-Ru Lou, Tai Chong Toh, Yee Han Tee, & Hanry Yu

Scientific Reports | 7:42816 | DOI: 10.1038/srep42816 2017

Wikipedia: Nuclear factor of activated T-cells (**NFAT**) is a general name applied to a family of transcription factors shown to be important in immune response. One or more members of the NFAT family is expressed in most cells of the immune system. NFAT is also involved in the development of cardiac, skeletal muscle, and nervous systems. The NFAT transcription factor family consists of five members NFATc1, NFATc2, NFATc3, NFATc4, and NFAT5. NFATc1 through NFATc4 are regulated by calcium signaling. Calcium signaling is critical to NFAT activation because calmodulin (CaM), a well-known calcium sensor protein, activates the serine/threonine phosphatase Calcineurin (CN). Activated CN rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in the amino termini of NFAT proteins, resulting in a conformational change that exposes a nuclear localization signal, resulting in NFAT nuclear import. Nuclear import of NFAT

proteins is opposed by maintenance kinases in the cytoplasm and export kinases in the nucleus. Export kinases, such as **PKA and GSK-3β, must be inactivated for NFAT nuclear retention**.

NFAT proteins have weak DNA-binding capacity. Therefore, to effectively bind DNA, NFAT proteins must cooperate with other nuclear resident transcription factors generically referred to as NFATn. This important feature of NFAT transcription factors enables integration and coincidence detection of calcium signals with other signaling pathways such as ras-MAPK or PKC. In addition, this signaling integration is involved in tissue-specific gene expression during development. A screen of ncRNA sequences identified in EST sequencing projects discovered a 'ncRNA repressor of the nuclear factor of activated T cells' called NRON.



At Emory: Geometric mean for serum parathyroid hormone (PTH) concentrations in patients with stage 3 and 4 chronic kidney disease treated with placebo or cholecalciferol, 50 000 IU once weekly, for 12 weeks. Serum PTH levels were measured in participants treated with placebo (*diamonds*) or cholecalciferol (*squares*) at baseline, 6 weeks, and 12 weeks of the study. The PTH levels of the 2 study groups are not significantly different at each of the 3 time points ($P = .14$). Error bars indicate 95% confidence intervals.

Abstract

OBJECTIVE: To investigate the efficacy of cholecalciferol (vitamin D3) in raising serum 25-hydroxyvitamin D (25[OH)]D) levels and reducing parathyroid hormone (PTH) levels in patients with chronic kidney disease (CKD).

METHODS: In this double-blind, randomized controlled pilot study, participants with CKD stage 3 and 4 (estimated glomerular filtration rate, 15-59 mL/min/1.73 m2), vitamin D insufficiency (serum 25[OH]D <30 ng/mL), and serum intact PTH levels >70 pg/mL were randomly assigned to receive either 50 000 IU of cholecalciferol or placebo once weekly for 12 weeks. Primary outcomes (25[OH]D and PTH levels) were measured at baseline, week 6, and week 12. Secondary outcomes (1,25-dihydroxvitamin D and bone turnover markers) were measured at baseline and week 12. Because of skewed data distribution, statistical analyses

were performed on a logarithmic scale. The difference between the group means was exponentiated to provide the geometric mean ratio. A linear mixed model using an unstructured variance-covariance matrix was used to examine change in the primary and secondary outcomes over time.

RESULTS: Geometric mean serum 25(OH)D concentrations of the study groups were similar at baseline (P = .77). At week 6, a significant difference between the treatment and placebo groups was detected (P = .001); this difference was maintained at week 12 (P = .002). Among cholecalciferol-treated participants, serum 25(OH)D concentration increased on average from 17.3 ng/mL (95% confidence interval [CI], 11.8-25.2) at baseline to 49.4 ng/mL (95% CI, 33.9-72.0) at week 12. As-treated analysis indicated a trend toward lower PTH levels among cholecalciferol-treated participants (p = 0.07).

CONCLUSION: Weekly cholecalciferol supplementation appears to be an effective treatment to correct vitamin D status in patients with CKD.

CHOLECALCIFEROL (VITAMIN D3) THERAPY AND VITAMIN D INSUFFICIENCY IN PATIENTS WITH CHRONIC KIDNEY DISEASE: A RANDOMIZED CONTROLLED PILOT STUDY

	Placebo				Cholecalciferol		
Analyte	Week 0	Week 6	Week 12	Week 0	Week 6	Week 12
Total Calcium mg/dL	9.0 (8.8–9.3) n=9		9.5 (9.3–9.6) n=10	9.1 (8.9–9.4) n=9		9.7 (9.5–9.8) n=10
25(OH)D ng/mL	18.6 (12.8–27.1) n=10	21.0 (15.7–28.2) n=9	19.5 (13.4–28.4) n=10	17.3 (11.8–25.2) n=10	44.5 (33.1–59.8) n=7	49.4 (33.9–72.0) n=10
PTH pg/mL	290.5 (179.7–469.8) n=10	335.1 (214.2–524.1) n=9	269.7 (153.3–474.5) n=10	288.9 (178.7–467.2) n=10	254.7 (161.4–401.9) n=7	200.5 (114.0–352.8) n=10
1,25(OH)2D pg/mL	29.7 (17.3–51.0)		30.8 (24.8–38.1)	29.1 (16.9–50.0)		40.2 (32.8–49.2)
BSAP IU/L	18.4 (10.2–33.3)		22.0 (19.3–25.0)	20.3 (13.4–30.5)		20.4 (18.0–23.1)
CTX pg/mL	0.36 (0.18–0.74)		0.24 (0.14–0.40)	0.34 (0.15–0.76)		0.29 (0.18–0.49)
TRAP5b pg/mL	2.9 (1.8–4.8)		3.5 (2.9–4.1)	3.8 (2.5–5.6)		3.2 (2.7–3.8)

CTX: serum C-telopeptide TRAP5b: tartarate-resistant acid phosphatase isoform 5b

Stage 3 & 4 CKD: placebo or cholecalciferol 50,000 IU weekly (10 per group)
Age ~60, 6 women/group, GFR ~30, Album ~3.6, Phos ~4.0, Ca x P ~38

Chandra P et al: Endocr Pract 14(1):10–17, 2008

Geometric mean for serum parathyroid hormone (PTH) concentrations in patients with stage 3 and 4 chronic kidney disease treated with placebo or cholecalciferol, 50 000 IU once weekly, for 12 weeks. Serum PTH levels were measured in participants treated with placebo (*diamonds*) or cholecalciferol (*squares*) at baseline, 6 weeks, and 12 weeks of the study. The PTH levels of the 2 study groups are not significantly different at each of the 3 time points ($P = .14$). Error bars indicate 95% confidence intervals.

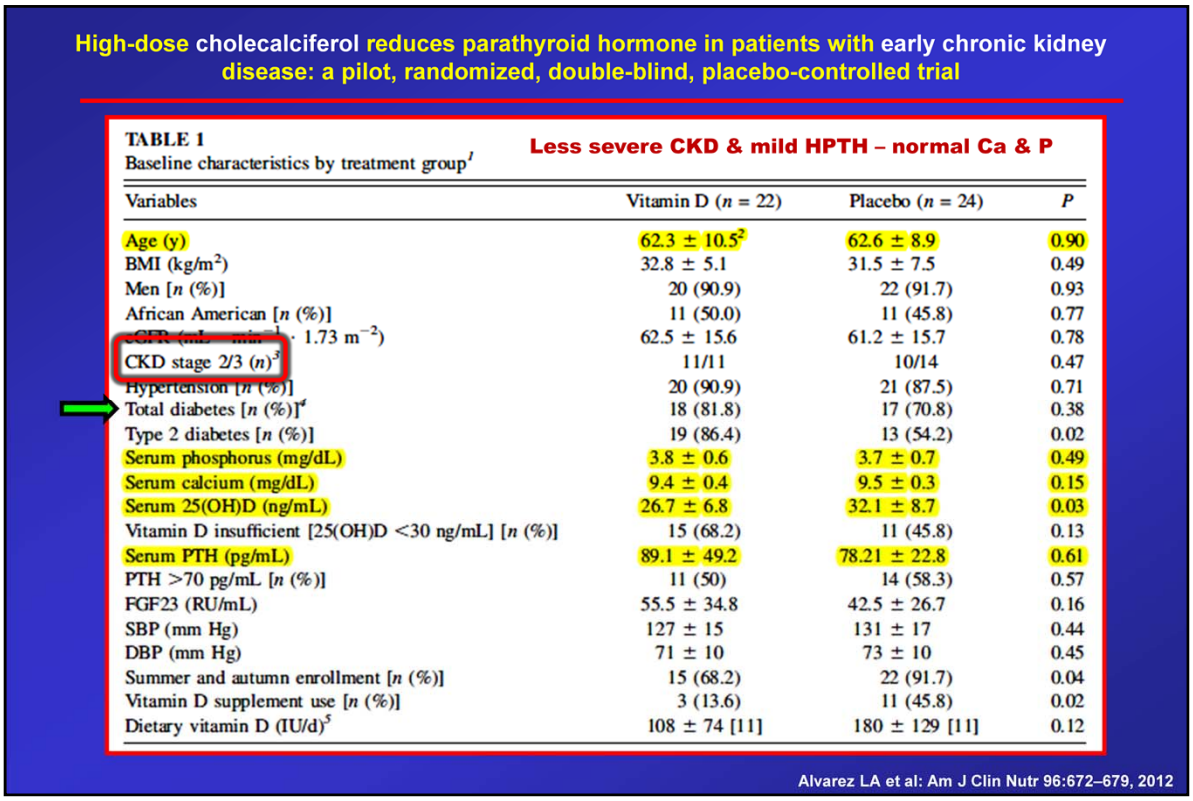


TABLE 1
Baseline characteristics by treatment group[1]

Variables	Vitamin D (n = 22)	Placebo (n = 24)	P
Age (y)	62.3 ± 10.5[2]	62.6 ± 8.9	0.90
BMI (kg/m^2)	32.8 ± 5.1	31.5 ± 7.5	0.49
Men [n (%)]	20 (90.9)	22 (91.7)	0.93
African American [n (%)]	11 (50.0)	11 (45.8)	0.77
GFR (mL · min^{-1} · 1.73 m^{-2})	62.5 ± 15.6	61.2 ± 15.7	0.78
CKD stage 2/3 (n)[3]	11/11	10/14	0.47
Hypertension [n (%)]	20 (90.9)	21 (87.5)	0.71
Total diabetes [n (%)][4]	18 (81.8)	17 (70.8)	0.38
Type 2 diabetes [n (%)]	19 (86.4)	13 (54.2)	0.02
Serum phosphorus (mg/dL)	3.8 ± 0.6	3.7 ± 0.7	0.49
Serum calcium (mg/dL)	9.4 ± 0.4	9.5 ± 0.3	0.15
Serum 25(OH)D (ng/mL)	26.7 ± 6.8	32.1 ± 8.7	0.03
Vitamin D insufficient [25(OH)D <30 ng/mL] [n (%)]	15 (68.2)	11 (45.8)	0.13
Serum PTH (pg/mL)	89.1 ± 49.2	78.21 ± 22.8	0.61
PTH >70 pg/mL [n (%)]	11 (50)	14 (58.3)	0.57
FGF23 (RU/mL)	55.5 ± 34.8	42.5 ± 26.7	0.16
SBP (mm Hg)	127 ± 15	131 ± 17	0.44
DBP (mm Hg)	71 ± 10	73 ± 10	0.45
Summer and autumn enrollment [n (%)]	15 (68.2)	22 (91.7)	0.04
Vitamin D supplement use [n (%)]	3 (13.6)	11 (45.8)	0.02
Dietary vitamin D (IU/d)[5]	108 ± 74 [11]	180 ± 129 [11]	0.12

Abstract

BACKGROUND: Vitamin D deficiency contributes to secondary hyperparathyroidism, which occurs early in chronic kidney disease (CKD).

OBJECTIVES: We aimed to determine whether high-dose cholecalciferol supplementation for 1 y in early CKD is sufficient to maintain optimal vitamin D status (serum 25-hydroxyvitamin D [25(OH)D] concentration ≥30 ng/mL) and decrease serum parathyroid hormone (PTH). A secondary aim was to determine the effect of cholecalciferol on blood pressure and serum fibroblast growth factor-23 (FGF23).

DESIGN: This was a double-blind, randomized, placebo-controlled trial. Forty-six subjects with early CKD (stages 2-3) were supplemented with oral cholecalciferol (vitamin D group; 50,000 IU/wk for 12 wk followed by 50,000 IU every other week for 40 wk) or a matching placebo for 1 y.

RESULTS: By 12 wk, serum 25(OH)D increased in the vitamin D group only [baseline (mean ± SD): **26.7** ± 6.8 to **42.8** ± 16.9 ng/mL; $P < 0.05$] and remained elevated at 1 y (group-by-time interaction: **$P < 0.001$**). PTH decreased from baseline only in the vitamin D group (baseline: **89.1** ± 49.3 to **70.1** ± 24.8 pg/mL; $P = 0.01$) at 12 wk, but values were not significantly different from baseline at 1 y

(75.4 ± 29.5 pg/mL; P = 0.16; group-by-time interaction: P = 0.09). Group differences were more pronounced in participants with secondary hyperparathyroidism (group-by-time interaction: P = 0.004). Blood pressure and FGF23 did not change in either group.

CONCLUSIONS: After 1 y, this oral cholecalciferol regimen was safe and sufficient to maintain serum 25(OH)D concentrations and prevent vitamin D insufficiency in early CKD. Furthermore, serum PTH improved after cholecalciferol treatment, particularly in patients who had secondary hyperparathyroidism.

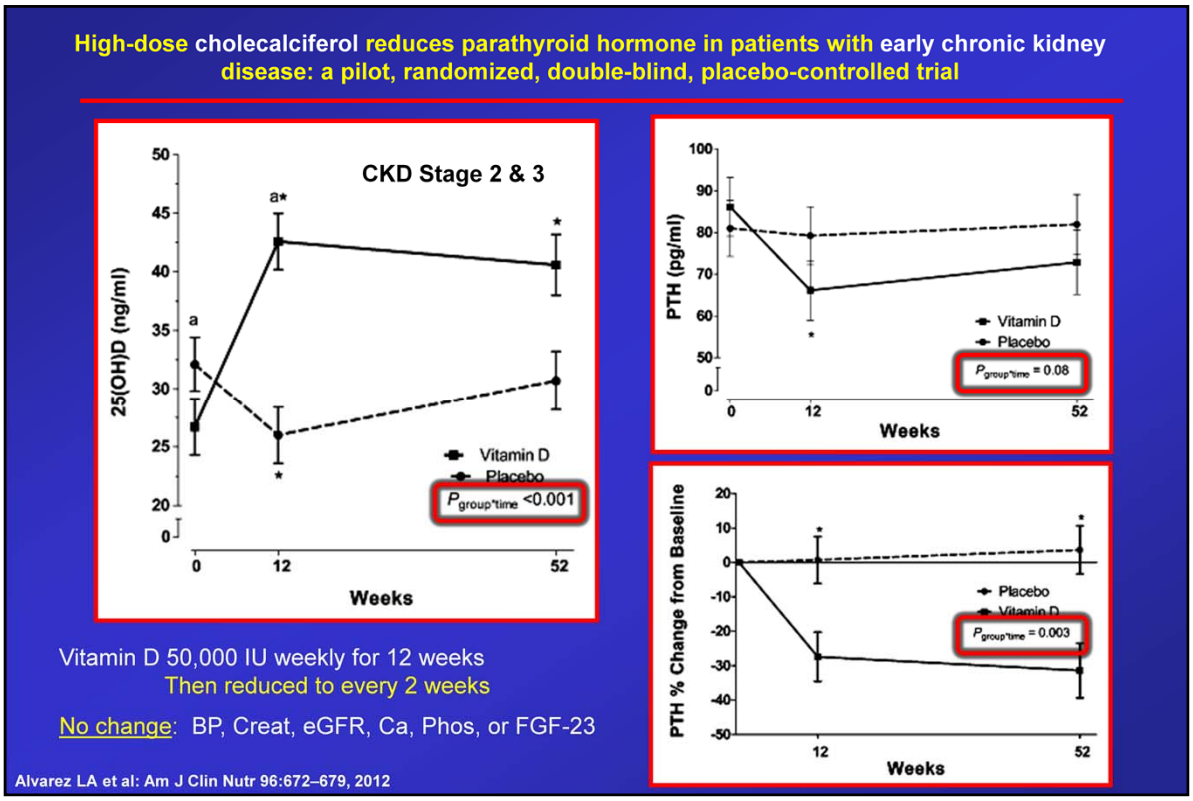
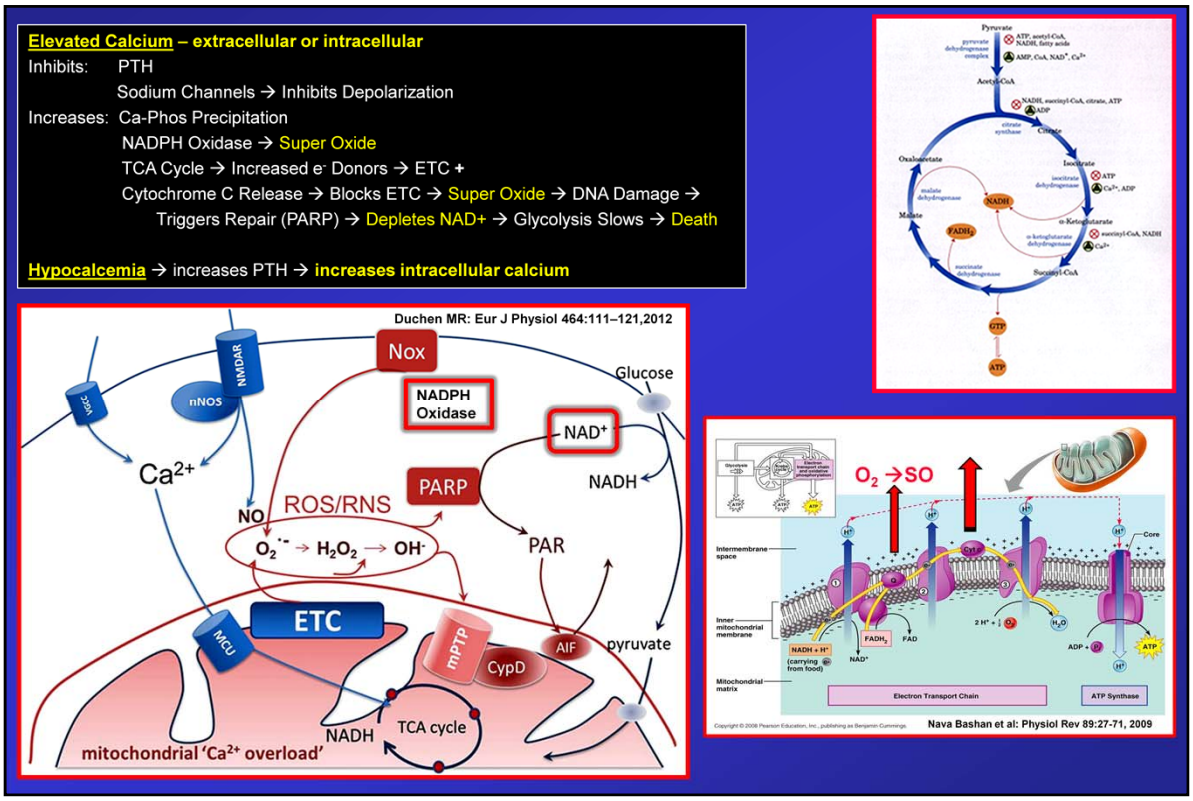


FIGURE 2. Least-squares mean (±SEM) serum 25(OH)D concentrations in early chronic kidney disease subjects who were randomly assigned to receive vitamin D or a placebo for 1 y. Subjects with early-stage chronic kidney disease (mean eGFR: 62 ± 15) were randomly assigned to receive 50,000 IU vitamin D/wk for 12 wk followed by 50,000 IU vitamin D every other week for 40 wk (n = 22) or an identically matched placebo (n = 24). Serum 25(OH)D concentrations are reported across time and by treatment group. The vitamin D group (solid line) had a significant increase in serum 25(OH)D by 12 wk, which remained elevated from baseline at 52 wk. The placebo group (dashed line) had a significant decrease in 25(OH)D by 12 wk. The group*time was determined with mixed-model repeated-measures ANOVA.

*Significant change from baseline, P , 0.05 (paired t test); a significant difference from placebo, P , 0.05 (t test). eGFR, estimated glomerular filtration rate; group*time, group-by-time interaction; 25(OH)D, 25-hydroxyvitamin D.



Mitochondria, calcium-dependent neuronal death and neurodegenerative disease. Eur J Physiol 464:111–121, 2012. Michael R. Duchen

Fig. 3 Scheme of pathways involved in glutamate-induced excitotoxicity. Calcium influx through voltage-gated or NMDAR-gated channels is followed by mitochondrial Ca2+ influx through the mitochondrial calcium uniporter (MCU). While the physiological consequence of raised intra-mitochondrial [Ca2+] is an increased activity of the three rate limiting enzymes of the TCA cycle, pathological and prolonged Ca2+ influx leads to mitochondrial Ca2+overload. NMDAR mediated Ca2+ influx is closely coupled to the generation of NO by nNOS; raised Ca2+ may activate the NADPH oxidase (Nox), while mitochondrial Ca2+ overload may also increase generation of superoxide by the electron transport chain (ETC). Nitrosative or oxidative stress arising either from the ETC or from Nox activation may cause over activation of PARP. PARP consumes NAD+ to form PAR polymers, causing depletion of NAD+, failure of glycolysis and so failure of mitochondrial substrate supply. This culminates in the loss of $\Delta\psi m$, ATP depletion and cell death. The PAR polymers generated by PARP may also cause release of AIF which amplifies cell death following its translocation to the nucleus.

Abstract Understanding the mechanisms of neuronal dysfunction and death represents a major frontier in contemporary medicine, involving the acute cell death

in stroke, and the attrition of the major neurodegenerative diseases, including Parkinson's, Alzheimer's, Huntington's and Motoneuron diseases. A growing body of evidence implicates mitochondrial dysfunction as a key step in the pathogenesis of all these diseases, with the promise that mitochondrial processes represent valuable potential therapeutic targets. Each disease is characterized by the loss of a specific vulnerable population of cells--dopaminergic neurons in Parkinson's disease, spinal motoneurons in Motoneuron disease, for example. We discuss the possible roles of cell type-specific calcium signalling mechanisms in defining the pathological phenotype of each of these major diseases and review central mechanisms of calcium-dependent mitochondrial-mediated cell death.

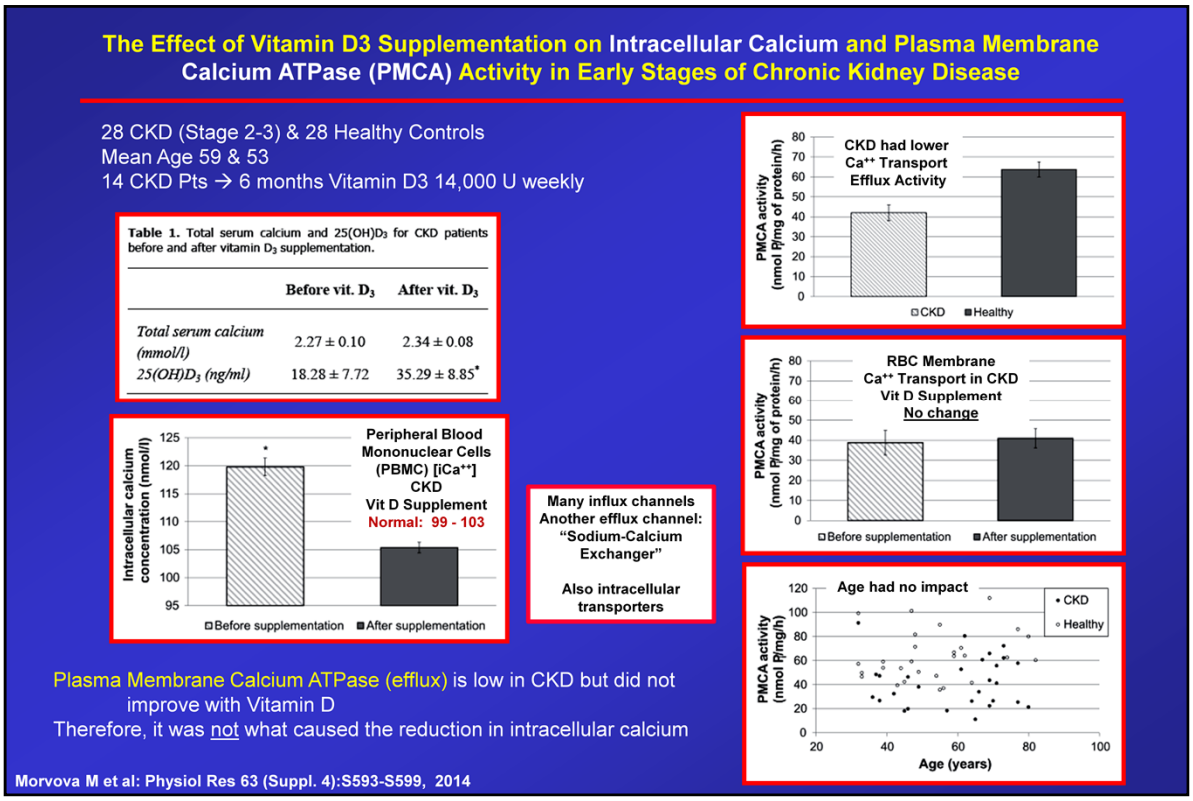


Fig. 1. Comparison of the mean values of free cytosolic calcium concentration (expressed in nmol/l) of PBMC in early stage CKD patients before and after 6 months vitamin D3 supplementation (* P<0.001, n=14). Data are given as mean ± SEM.

Fig. 2. Comparison of the mean values of PMCA activity of RBC membrane (expressed in nmol of inorganic phosphate (Pi) produced per mg of plasma membrane protein per hour) in early stage CKD patients before and after 6 months vitamin D3 supplementation (n=14). Data are given as mean ± SEM.

Fig. 3. Comparison of the mean values of PMCA activity of RBCs membrane (expressed in nmol of inorganic phosphate (Pi) produced per mg of plasma membrane protein per hour) in early stage CKD patients and healthy donors (* P<0.001, n=28). Data are given as mean ± SEM.

Fig. 4. The dependence of PMCA activity on age of CKD patients (□) and healthy donors (○) (n=28). No significant correlation was detected. These results indicate that vitamin D3 supplementation had a lowering effect on [Ca2+]i and negligible effect on PMCA activity in CKD patients.

Abstract

Chronic kidney disease (CKD) is associated with increased concentration of intracellular calcium, which is pathological and may lead to irreversible damage of cell functions and structures. The aim of our study was to investigate the impact of 6 months vitamin D(3) supplementation (14 000 IU/week) on free cytosolic calcium concentration ([Ca(2+)](i)) and on the plasma membrane calcium ATPase (PMCA) activity of patients with CKD stage 2-3.

PMCA activity of patients was also compared to that of healthy volunteers. Vitamin D(3) supplementation of CKD patients resulted in the decrease of **[Ca(2+)](i) (119.79**+/-5.87 nmol/l vs. **105.36**+/-3.59 nmol/l, n=14, P<0.001), whereas PMCA activity of CKD patients (38.75+/-22.89 nmol P(i)/mg/h) remained unchanged after vitamin D(3) supplementation (40.96+/-17.74 nmol P(i)/mg/h, n=14). PMCA activity of early stage CKD patients before supplementation of vitamin D(3), was reduced by 34% (42.01+/-20.64 nmol P(i)/mg/h) in comparison to healthy volunteers (63.68+/-20.32 nmol P(i)/mg/h, n=28, P<0.001).

These results indicate that vitamin D(3) supplementation had a **lowering effect on [Ca(2+)]**(i) and negligible effect on PMCA activity in CKD patients.

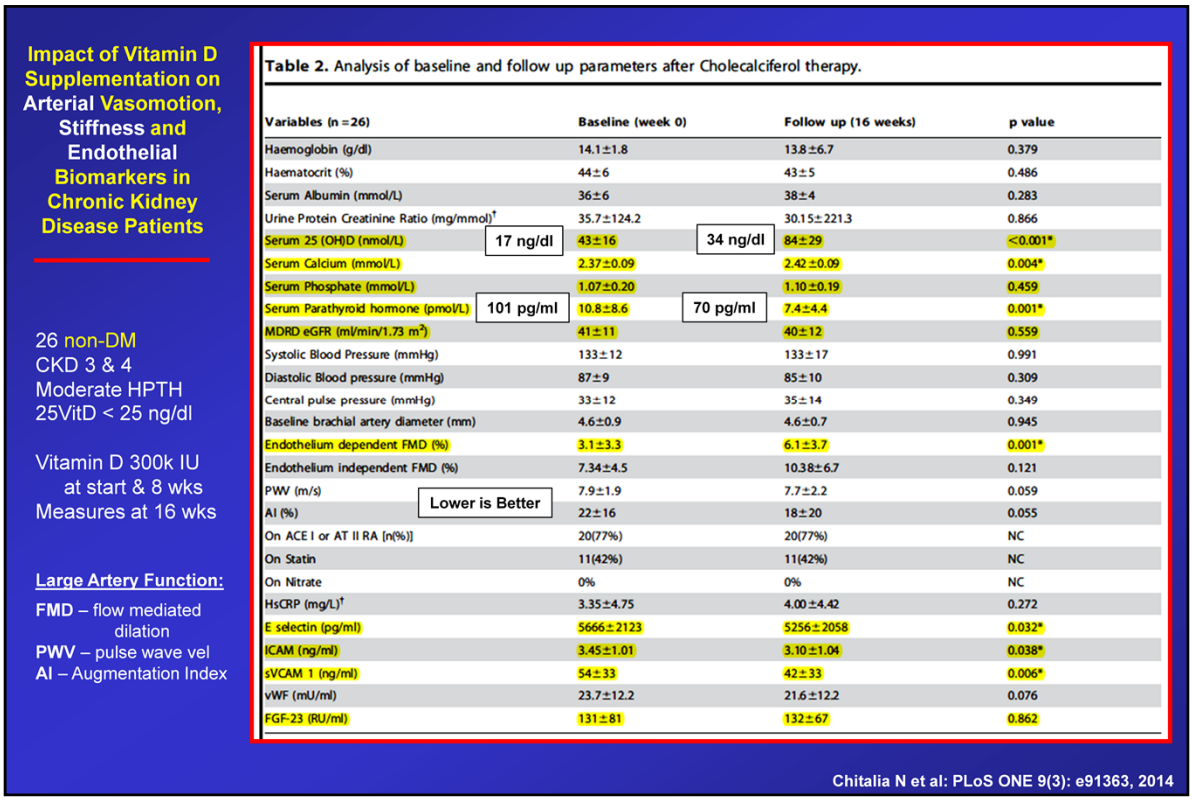


Table 2. Analysis of baseline and follow up parameters after Cholecalciferol therapy.

Variables (n =26)	Baseline (week 0)	Follow up (16 weeks)	p value
Haemoglobin (g/dl)	14.1±1.8	13.8±6.7	0.379
Haematocrit (%)	44±6	43±5	0.486
Serum Albumin (mmol/L)	36±6	38±4	0.283
Urine Protein Creatinine Ratio (mg/mmol)†	35.7±124.2	30.15±221.3	0.866
Serum 25 (OH)D (nmol/L)	43±16	84±29	<0.001*
Serum Calcium (mmol/L)	2.37±0.09	2.42±0.09	0.004*
Serum Phosphate (mmol/L)	1.07±0.20	1.10±0.19	0.459
Serum Parathyroid hormone (pmol/L)	10.8±8.6	7.4±4.4	0.001*
MDRD eGFR (ml/min/1.73 m²)	41±11	40±12	0.559
Systolic Blood Pressure (mmHg)	133±12	133±17	0.991
Diastolic Blood pressure (mmHg)	87±9	85±10	0.309
Central pulse pressure (mmHg)	33±12	35±14	0.349
Baseline brachial artery diameter (mm)	4.6±0.9	4.6±0.7	0.945
Endothelium dependent FMD (%)	3.1±3.3	6.1±3.7	0.001*
Endothelium independent FMD (%)	7.34±4.5	10.38±6.7	0.121
PWV (m/s)	7.9±1.9	7.7±2.2	0.059
AI (%)	22±16	18±20	0.055
On ACE I or AT II RA [n(%)]	20(77%)	20(77%)	NC
On Statin	11(42%)	11(42%)	NC
On Nitrate	0%	0%	NC
HsCRP (mg/L)†	3.35±4.75	4.00±4.42	0.272
E selectin (pg/ml)	5666±2123	5256±2058	0.032*
ICAM (ng/ml)	3.45±1.01	3.10±1.04	0.038*
sVCAM 1 (ng/ml)	54±33	42±33	0.006*
vWF (mU/ml)	23.7±12.2	21.6±12.2	0.076
FGF-23 (RU/ml)	131±81	132±67	0.862

Abstract

BACKGROUND: Cardiovascular events are frequent and vascular endothelial function is abnormal in patients with chronic kidney disease (CKD). We demonstrated endothelial dysfunction with vitamin D deficiency in CKD patients; however the impact of cholecalciferol supplementation on vascular stiffness and vasomotor function, endothelial and bone biomarkers in CKD patients with low 25-hydroxy vitamin D [25(OH)D] is unknown, which this study investigated.

Methods: We assessed non-diabetic patients with CKD stage 3/4, age 17–80 years and serum 25(OH)D ,75 nmol/L. Brachial artery Flow Mediated Dilation (FMD), Pulse Wave Velocity (PWV), Augmentation Index (AI) and circulating blood biomarkers were evaluated at baseline and at 16 weeks. Oral 300,000 units cholecalciferol was administered at baseline and 8-weeks.

Results: Clinical characteristics of 26 patients were: age 50±14 (mean±1SD) years, eGFR 41±11 ml/min/1.73 m2, males 73%, dyslipidaemia 36%, smokers 23% and hypertensives 87%. At 16-week serum 25(OH)D and calcium increased (43±16 to 84±29 nmol/L, p = 0.001 and 2.37±0.09 to 2.42±0.09 mmol/L; p = 0.004, respectively) and parathyroid hormone decreased (10.8±8.6 to 7.4±4.4; p = 0.001). FMD improved from 3.1±3.3% to 6.1±3.7%, p = 0.001. Endothelial biomarker concentrations decreased: E-Selectin from 5666±2123 to 5256±2058 pg/mL; p =

0.032, ICAM-1, 3.45±0.01 to 3.10±1.04 ng/mL; p = 0.038 and VCAM-1, 54±33 to 42±33 ng/mL; p = 0.006. eGFR, BP, PWV, AI, hsCRP, von Willebrand factor and Fibroblast Growth Factor-23, remained unchanged.

Conclusion: This study demonstrates for the first time improvement of endothelial vasomotor and secretory functions with vitamin D in CKD patients without significant adverse effects on arterial stiffness, serum calcium or FGF-23.

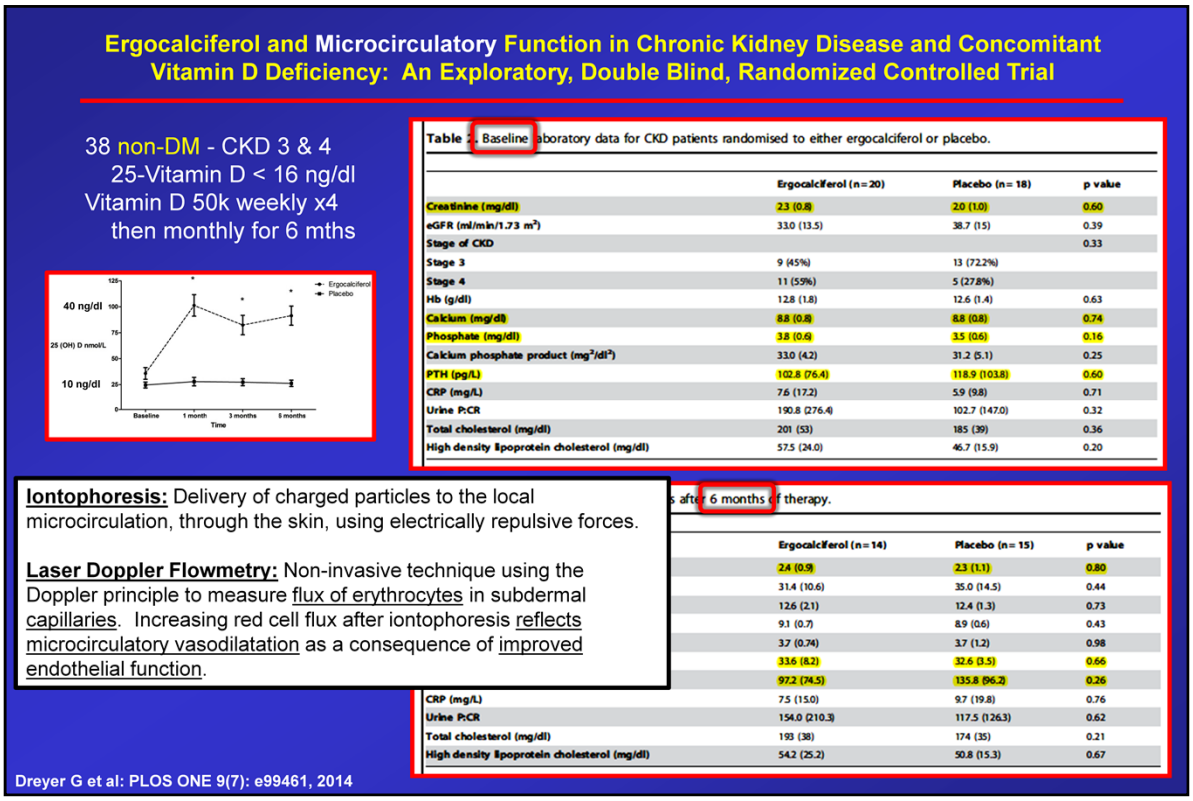


Figure 2. 25 (OH) Vitamin D levels in patients treated with ergocalciferol and placebo. Bonferroni post tests following two way repeated measures ANOVA at 1,3 and 6 months p=0.0001.

(* = statistically significant).

Figure 3. Percentage rise from baseline flux in arbitrary units (AU) after iontophoresis of ACh. Absolute values of percentage change influx (AU):

baseline - ergocalciferol 964.8, placebo 785.9 (p = NS).

1 month - ergocalciferol 979.5, placebo 690.9 (p = NS).

3 months – ergocalciferol 543.7, placebo 613.5 (p = NS).

6 months – ergocalciferol 1130.0, placebo 540.6 (p = 0.012).

p values are Bonferroni post test following two way repeated measures ANOVA. (* = statistically significant).

Abstract BACKGROUND AND OBJECTIVES: Vitamin D deficiency and endothelial dysfunction are non-traditional risk factors for cardiovascular events in chronic kidney disease. Previous studies in chronic kidney disease have failed to demonstrate a beneficial effect of vitamin D on arterial stiffness, left ventricular mass and inflammation but none have assessed the effect of vitamin D on microcirculatory endothelial function.

STUDY DESIGN: We conducted a randomized controlled trial of 38 patients with non diabetic chronic kidney disease stage 3-4 and concomitant vitamin D deficiency (<16 ng/dl) who received oral ergocalciferol (50,000 IU weekly for one month followed by 50,000 IU monthly) or placebo over 6 months. The primary outcome was change in microcirculatory function measured by laser Doppler flowmetry after iontophoresis of acetylcholine. Secondary endpoints were tissue advanced glycation end products, sublingual functional capillary density and flow index as well as macrovascular parameters. Parallel 'in vitro' experiments were conducted to determine the effect of ergocalciferol on cultured human endothelial cells.

RESULTS: Twenty patients received ergocalciferol and 18 patients received placebo. After 6 months, there was a significant improvement in the ergocalciferol group in both endothelium dependent microcirculatory vasodilatation after iontophoresis of acetylcholine ($p=0.03$) and a reduction in tissue advanced glycation end products ($p=0.03$). There were no changes in sublingual microcirculatory parameters. Pulse pressure ($p=0.01$) but not aortic pulse wave velocity was reduced. There were no significant changes in bone mineral parameters, blood pressure or left ventricular mass index suggesting that ergocalciferol improved endothelial function independently of these parameters. In parallel experiments, expression of endothelial nitric oxide synthase and activity were increased in human endothelial cells in a dose dependent manner.

CONCLUSIONS: Ergocalciferol improved microcirculatory endothelial function in patients with chronic kidney disease and concomitant vitamin D deficiency. This process may be mediated through enhanced expression and activity of endothelial nitric oxide synthase.

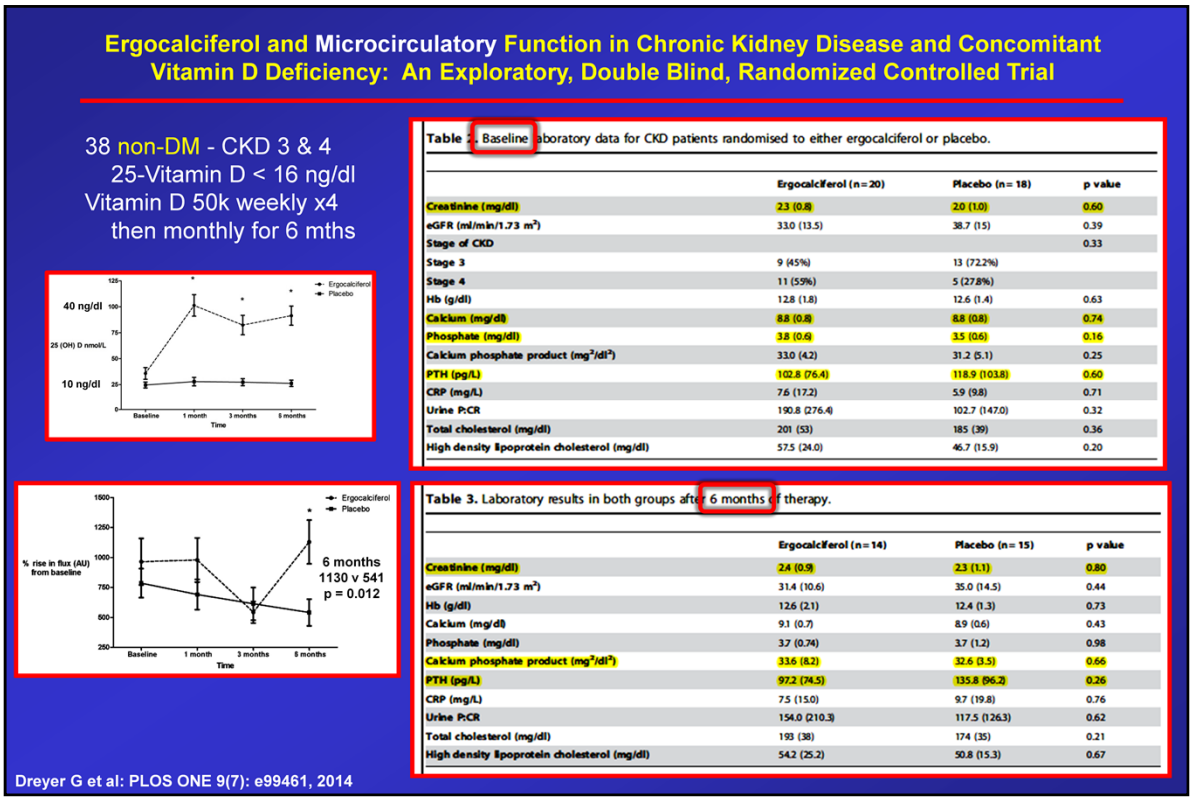


Figure 2. 25 (OH) D levels in patients treated with ergocalciferol and placebo. Bonferroni post tests following two way repeated measures ANOVA at 1,3 and 6 months p=0.0001.

(* = statistically significant).

Figure 3. Percentage rise from baseline flux in arbitrary units (AU) after iontophoresis of ACh. Absolute values of percentage change influx (AU): baseline - ergocalciferol 964.8, placebo 785.9 (p = NS). 1 month - ergocalciferol 979.5, placebo 690.9 (p = NS). 3 months – ergocalciferol 543.7, placebo 613.5 (p = NS). 6 months – ergocalciferol 1130.0, placebo 540.6 (p = 0.012). p values are Bonferroni post test following two way repeated measures ANOVA. (* = statistically significant).

Abstract

BACKGROUND AND OBJECTIVES: Vitamin D deficiency and endothelial dysfunction are non-traditional risk factors for cardiovascular events in chronic kidney disease. Previous studies in chronic kidney disease have failed to demonstrate a beneficial effect of vitamin D on arterial stiffness, left ventricular mass and inflammation but none have assessed the effect of vitamin D on microcirculatory endothelial function.

STUDY DESIGN: We conducted a randomised controlled trial of 38 patients with non diabetic chronic kidney disease stage 3-4 and concomitant vitamin D deficiency (<16 ng/dl) who received oral ergocalciferol (50,000 IU weekly for one month followed by 50,000 IU monthly) or placebo over 6 months. The primary outcome was change in microcirculatory function measured by laser Doppler flowmetry after iontophoresis of acetylcholine. Secondary endpoints were tissue advanced glycation end products, sublingual functional capillary density and flow index as well as macrovascular parameters. Parallel in vitro experiments were conducted to determine the effect of ergocalciferol on cultured human endothelial cells.

RESULTS: Twenty patients received ergocalciferol and 18 patients received placebo. After 6 months, there was a significant improvement in the ergocalciferol group in both endothelium dependent microcirculatory vasodilatation after iontophoresis of acetylcholine ($p=0.03$) and a reduction in tissue advanced glycation end products ($p=0.03$). There were no changes in sublingual microcirculatory parameters. Pulse pressure ($p=0.01$) but not aortic pulse wave velocity was reduced. There were no significant changes in bone mineral parameters, blood pressure or left ventricular mass index suggesting that ergocalciferol improved endothelial function independently of these parameters. In parallel experiments, expression of endothelial nitric oxide synthase and activity were increased in human endothelial cells in a dose dependent manner.

CONCLUSIONS: Ergocalciferol improved microcirculatory endothelial function in patients with chronic kidney disease and concomitant vitamin D deficiency. This process may be mediated through enhanced expression and activity of endothelial nitric oxide synthase.

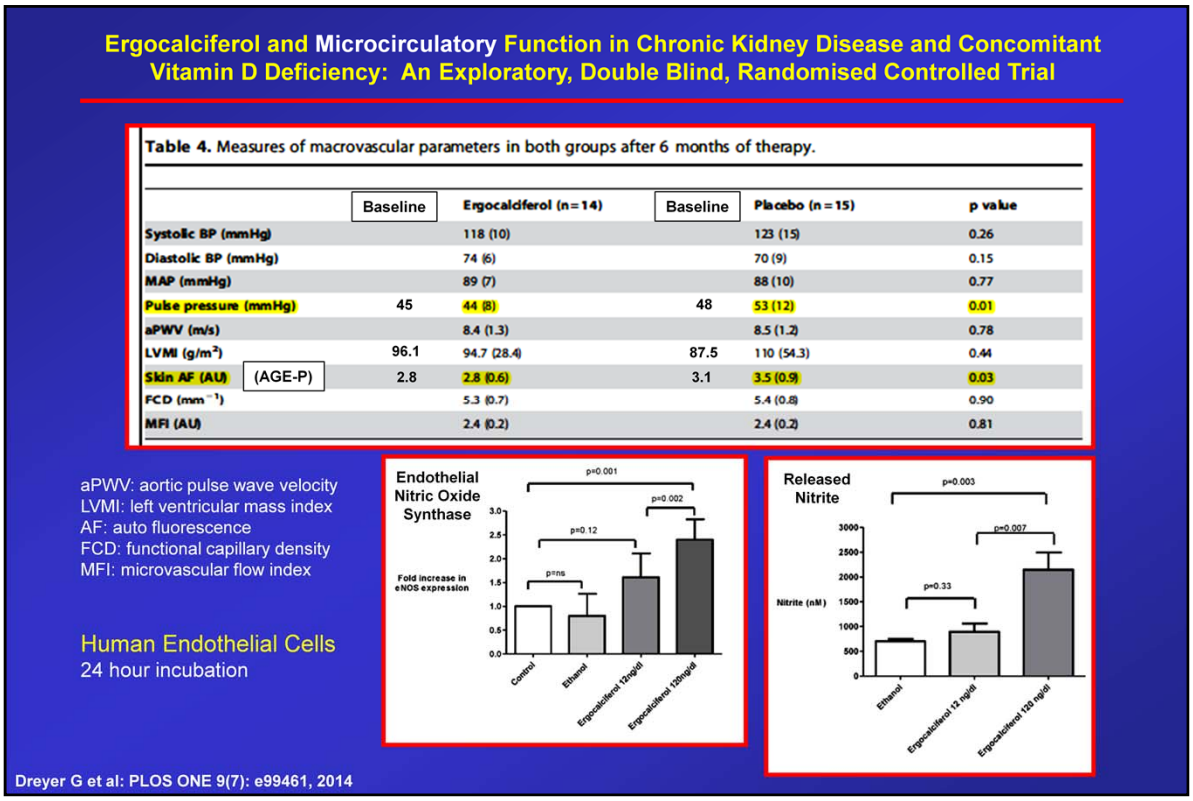


Figure 5. Fold increase in eNOS expression by RT-PCR in cultured HAEC.

Figure 6. Nitrite levels in supernatants of HAEC. Cultured in low dose (12 ng/dl) and high dose (120 ng/dl) ergocalciferol after 24 h incubation.

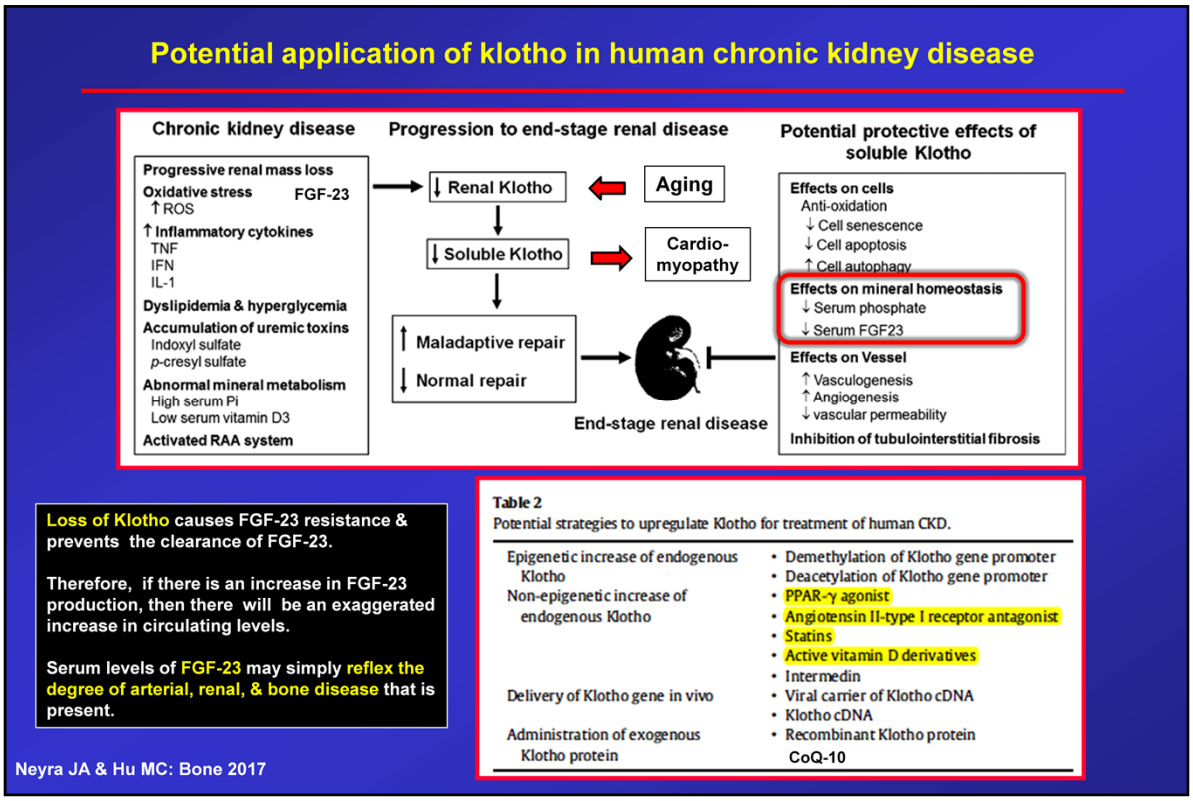


Fig. 2. Potential mechanisms of Klotho downregulation in CKD, and beneficial effects of soluble Klotho on CKD. Left panel: Loss of renal mass, over production of reactive oxygen species (ROS) as well as pro-inflammatory cytokines including tumor necrosis factor (TNF), interferon (IFN) and interleukin 1 (IL-1), dyslipidemia and hyperglycemia, and elevation of uremic toxins including indoxyl sulfate and p-cresyl sulfate may contribute to or participate in downregulation of renal Klotho. Furthermore, high serum phosphate and FGF23 as well as low serum 1,25-Vit.D3 inhibit renal Klotho expression. Low serum 1,25-Vit.D3 not only reduces Klotho expression, but also stimulates renin-aldosterone-angiotensin (RAA) system which further suppresses Klotho production. Middle panel: Reduced Klotho expression in the kidney would lead to endocrine Klotho deficiency in CKD. Low soluble Klotho promotes CKD progression to ESRD through impaired normal renal repair process and induction of maladaptive repair process. Right panel: Supplementation of soluble Klotho protein retards CKD progression through multiple biologic actions: (1) cytoprotection via anti-oxidation, reduction of cell senescence and apoptosis, and upregulation of autophagy, hence accelerating renal tubule regeneration; (2) correction of high serum phosphate and FGF23; (3) maintenance of peritubular capillary formation and function; and (4) inhibition of tubulo-interstitial fibrosis.

Abstract The extracellular domain of transmembrane alpha-Klotho (αKlotho, hereinafter simply called Klotho) is cleaved by secretases and released into the circulation as soluble Klotho. Soluble Klotho in the circulation starts to decline

early in chronic kidney disease (CKD) stage 2 and urinary Klotho possibly even earlier in CKD stage 1. Therefore soluble Klotho could serve as an early and sensitive marker of kidney function decline. Moreover, preclinical animal data support Klotho deficiency is not just merely a biomarker, but a pathogenic factor for CKD progression and extra-renal CKD complications including cardiovascular disease and disturbed mineral metabolism. Prevention of Klotho decline, re-activation of endogenous Klotho production or supplementation of exogenous Klotho are all associated with attenuation of renal fibrosis, retardation of CKD progression, improvement of mineral metabolism, amelioration of cardiomyopathy, and alleviation of vascular calcification in CKD. Therefore Klotho is not only a diagnostic and/or prognostic marker for CKD, but the treatment of Klotho deficiency may be a promising strategy to prevent, retard, and decrease the burden of comorbidity in CKD.

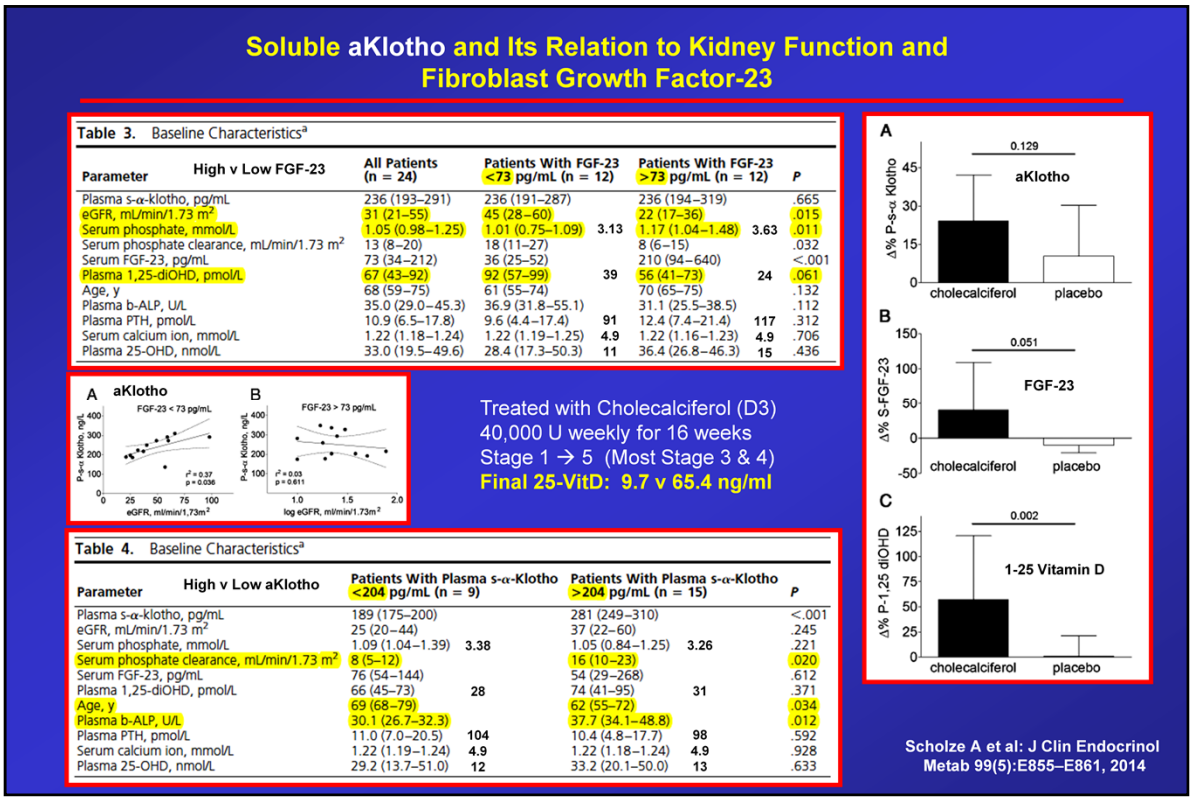


Figure 1. A and B, Association of plasma (P-) s-aklotho and eGFR according to FGF-23 concentrations below (A) and above (B) the median (73 pg/mL). In the group with lower FGF-23 values (A), analysis showed a significant correlation between s-a-klotho and eGFR. Regression line and 95% confidence interval are shown. The s-a-klotho concentrations in the group with FGF-23 above median (B) were normally distributed after logarithmic transformation. There was no significant association with eGFR.

Figure 2. Comparison of percent changes during the 8-week study period in the cholecalciferol and placebo group.

A, The percent change (%) of plasma (P-) s-aklotho was not significantly different between both groups.

B, Percent change of FGF-23 showed a borderline significance between the groups.

C, The percent change of 1,25-diOHD was significantly different between the groups.

Mann-Whitney *U* test was used.

Abstract

CONTEXT: Relations between fibroblast growth factor-23 (FGF-23), soluble α-klotho (s-α-klotho), and kidney function in chronic kidney disease (CKD) are still

unclear. Especially the role of s-α-klotho requires further study.

OBJECTIVES: Our objectives were to analyze the relation of s-α-klotho to estimated glomerular filtration rate (eGFR), FGF-23, and other parameters of calcium-phosphate metabolism and to investigate the response of s-α-klotho to cholecalciferol.

PATIENTS, DESIGN, AND SETTING: Twenty-four CKD (stage 1-5) patients participated in this 8-week randomized controlled trial (vitamin D and chronic renal insufficiency).

INTERVENTIONS: Interventions included 40 000 IU cholecalciferol or placebo weekly.

MAIN OUTCOME MEASURE: S-α-klotho was determined by ELISA with antihuman klotho antibodies 67G3 and 91F1.

RESULTS: For all patients, s-α-klotho concentrations did not differ between CKD stages. When patients were subdivided based on FGF-23 concentrations, a positive association of s-α-klotho with eGFR became apparent in patients with lower than median FGF-23 concentrations but not in those above median value. Patients with s-α-klotho below 204 pg/mL showed higher age, lower phosphate clearance, and lower bone-specific alkaline phosphatase compared with patients with higher s-α-klotho. Treatment with cholecalciferol significantly increased 1,25-dihydroxyvitamin D. The increase of FGF-23 had only borderline significance. There was no significant effect of high-dose cholecalciferol administration for 8 weeks on plasma s-α-klotho.

CONCLUSIONS: CKD patients with s-α-klotho below 204 pg/mL had higher age, lower phosphate clearance, and lower bone-specific alkaline phosphatase. An association of s-α-klotho with eGFR was observed only in the presence of close to normal, but not high, FGF-23 concentrations. Cholecalciferol treatment did not change s-α-klotho concentrations.

Calcitriol Supplementation

Ergocalciferol versus calcitriol for controlling chronic kidney disease mineral bone disorder in stage 3 to 5 CKD: A randomized controlled trial

Age ~57 Female 32%

DM 30%, GN 20%, HT 12%

Stage 3: 77% 4: 18% 5: 8%
No difference in cardiac or renal endpoints

Ergocalciferol: 50,000 IU weekly for 3 months, then monthly
Calcitriol: 0.25 mcg daily → titrated based on Ca^{++}, Phos, & PTH
(Average final calcitriol dose not given)
GFR (lower), Ca^{++}, & Phos (higher) – same changes in both groups
TGs & AlkPhos lower; 25VitD higher w/ Ergocalciferol
PTH trending in opposite directions (lower w/ Ergo)

Table 2
Baseline and final biochemical parameters changes. Followed for ~33 months

Parameters	Group VitD2	(Ergocal: 104 pts)		Group VitD3	(Calcitriol: 100 pts)		Between-treatment group P	
Routine laboratory data	Baseline	Final	Pre-Post P, t	Baseline	Final	Pre-Post P, t	Baseline P, t	Final P, t
Hemoglobin, g/L	134.2 ± 18.6	132.3 ± 24.9	0.263, 1.126	136.0 ± 19.3	134.1 ± 24.9	0.262, 1.129	0.413, −0.820	0.624, −0.492
hsCRP, mg/L	3.88 ± 8.30	2.85 ± 4.97	0.281, 1.084	2.78 ± 3.09	3.11 ± 4.44	0.479, −0.711	0.229, 1.207	0.574, −0.563
Creatinine, mg/dl	182.4 ± 109.0	244.1 ± 234.3	< 0.001[a], −4.488	173.6 ± 120.3	216.1 ± 183.0	< 0.001[b], −3.758	0.586, 0.545	0.344, 0.949
Urea nitrogen, mg/dl	10.39 ± 4.66	12.69 ± 7.25	< 0.001[a], −4.984	9.94 ± 5.09	12.31 ± 7.57	< 0.001[b], −4.108	0.474, 0.718	0.712, 0.370
Uric acid, mg/dl	372.8 ± 97.7	371.3 ± 88.6	0.901, 0.124	364.6 ± 73.2	389.6 ± 95.8	0.028[c], −2.232	0.501, 0.675	0.159, −1.412
Albumin, g/dl	41.29 ± 3.30	42.29 ± 3.84	0.004[a], −2.942	41.78 ± 2.77	42.71 ± 3.58	0.009[b], −2.651	0.255, −1.142	0.426, −0.798
Ferritin, mg/dl	117.8 ± 90.9	106.2 ± 88.2	0.174, 1.373	121.5 ± 92.7	115.8 ± 109.2	0.431, 0.790	0.753, 0.315	0.447, −0.763
Total cholesterol, mg/dl	4.99 ± 0.90	4.70 ± 0.95	0.008[a], 2.702	5.15 ± 0.86	4.78 ± 0.98	0.001[b], 3.540	0.181, −1.342	0.540, −0.613
Triglycerides, mg/dl	1.88 ± 1.02	1.58 ± 0.70	0.003[a], 2.995	2.10 ± 1.44	2.03 ± 1.76	0.643, 0.465	0.201, −1.282	0.017[d], −2.397
CKD-MBD biomarkers								
Calcium, mg/dl	2.29 ± 0.14	2.31 ± 0.14	0.117, −1.581	2.31 ± 0.11	2.33 ± 0.14	0.309, −1.023	0.129, −1.523	0.554, −0.593
Phosphorus, mg/dl	1.15 ± 0.24	1.27 ± 0.39	< 0.001[a], −3.715	1.14 ± 0.21	1.25 ± 0.33	< 0.001[b], −4.050	0.627, 0.487	0.694, 0.394
Alkaline phosphatase, U/L	81.92 ± 20.71	76.78 ± 21.23	0.003[a], 3.092	83.78 ± 19.41	82.04 ± 24.19	0.399, 0.846	0.508, −0.663	0.100, −1.653
Intact PTH, pg/ml	109.7 ± 127.8	96.5 ± 87.1	0.179, 1352	89.5 ± 75.0	108.1 ± 134.0	0.106, −1.633	0.173, 1.367	0.463, −0.736
25(OH)D2, ng/ml	3.97 ± 2.22	29.53 ± 12.02	< 0.001[a], 4.584	3.98 ± 2.85	4.40 ± 4.80	0.447, −0.763	0.996, 0.005	< 0.001[e], −19.456
25(OH)D3, ng/ml	11.17 ± 5.71	7.79 ± 5.62	< 0.001[a], 4.58	10.93 ± 4.61	13.68 ± 6.53	< 0.001[b], −3.778	0.737, −0.336	< 0.001[e], 6.664
25(OH)D, ng/ml	15.14 ± 7.46	37.32 ± 10.49	< 0.001[a], −19.692	14.90 ± 6.15	18.08 ± 7.55	< 0.001[b], −3.820	0.800, −0.253	< 0.001[e], −14.982

Zhang D et al: Euro J Pharm 789:127–133, 2016

Maintenance target levels of serum calcium, phosphorus, and intact parathyroid hormone as the primary outcome measure did not show significant difference in frequencies between the two groups. In summary, treatment of CKD-mineral and bone disorders in CKD patients at stages 3 to 5 using ergocalciferol has a similar long-term efficacy and safety profile as calcitriol.

a There are very significant differences (P<0.01) between baseline and final values in group VitD2 by paired-samples t test.

b There are very significant differences (P<0.01) between baseline and final values in group VitD3 by paired-samples t test.

c There are significant differences (P<0.05) between baseline and final values in group VitD3 by paired-samples t test.

d There are very significant differences (P<0.01) between group VitD2 and group VitD3 on final values by independent-samples t test.

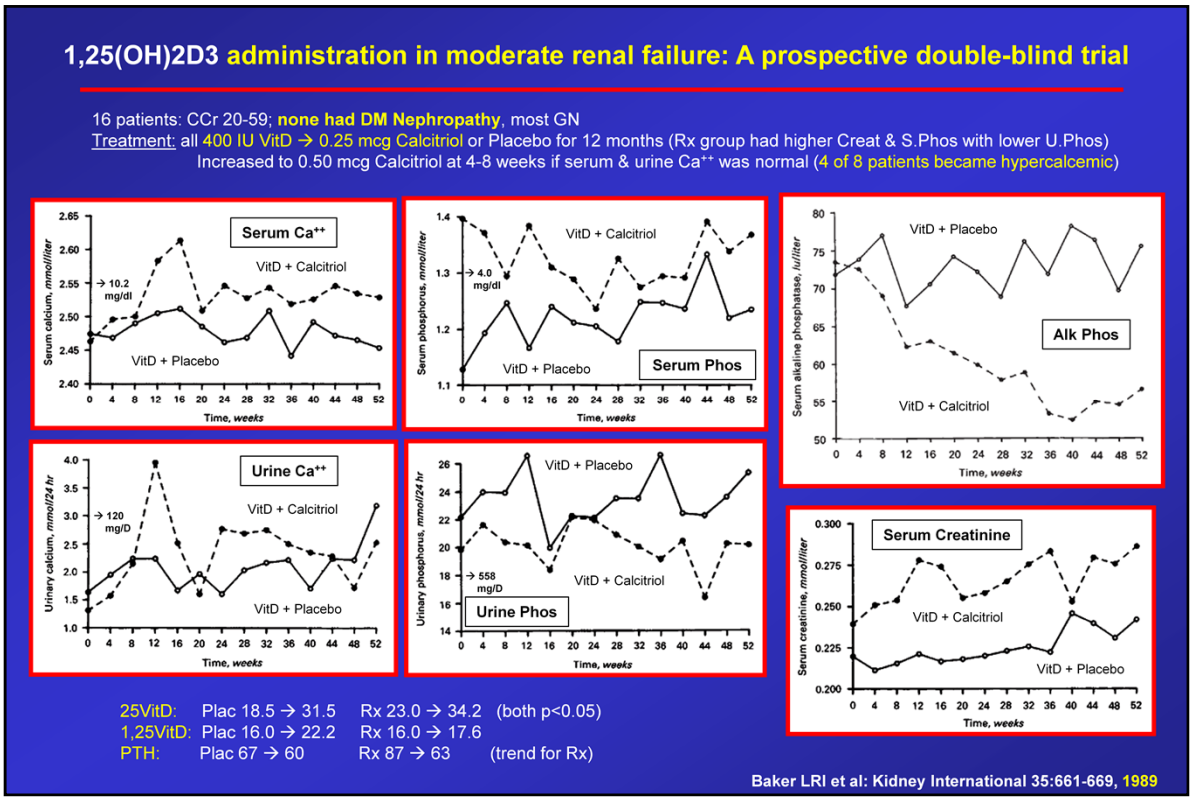
e There are significant differences (P<0.01) between group VitD2 and group VitD3 on final values by independent-samples t test.

Abstract To compare the efficacy and safety of ergocalciferol and calcitriol in stage 3 to 5 chronic kidney disease (CKD) patients, a randomized, prospective, controlled, open-labeled study was designed.

204 patients were enrolled into the present study with following-up duration of 33.2±3.8 months. Patients in Group VitD2 (n=104) and Group aVitD3 (n=100) were treated by ergocalciferol and calcitriol, respectively.

The 25-hydroxyvitamin D levels of group VitD2 increased significantly from 15.14±7.46 to 37.32±10.49 ng/ml ($P<0.001$, $t=-19.692$) and increased more ($P<0.001$, $t=-14.982$) than those of group aVitD3, which increased from 14.90±6.15 to 18.08±7.55 ng/ml. Maintenance target levels of serum calcium, phosphorus, and intact parathyroid hormone as the primary outcome measure did not show significant difference in frequencies between two groups.

In summary, treatment of CKD-mineral and bone disorders in CKD patients at stages 3 to 5 using ergocalciferol has a similar long-term efficacy and safety profile as calcitriol.



This study represents the first randomized prospective, double-blind, placebo-controlled trial of the efficacy of 1,25(OH),D3 on bone histology and serum biochemistry in patients with mild to moderate renal failure. Sixteen patients with chronic renal impairment (creatinine clearance 20 to 59 ml per mm) received either l,25(OH)2D3, at a dose of 0.25 to 0.5 g daily (eight patients), or placebo. Transiliac crest bone biopsies were performed before entrance into the study and after 12 months of experimental observation. None of the patients were symptomatic or had radiological evidence of bone disease.

Of the thirteen patients who completed the study, initial serum 1 ,25(OH)2D levels were low in seven patients and parathyroid hormone levels were elevated in seven patients. **Bone histology was abnormal in all patients**. 1,25(OH),D treatment was associated with a significant fall in serum phosphorus and alkaline phosphatase concentrations as well as with **histological evidence of an amelioration of hyperparathyroid changes**. In contrast to previous reports, no deterioration of renal function attributable to the treatment occurred, perhaps because a modest dose of l,25(OH)2D3 was employed combined with meticulous monitoring. Further investigation is required to determine whether alternative therapeutic strategies (smaller doses or intermittent therapy) may avoid the potential for suppressing bone turnover to abnormally low levels in the long term.

1,25(OH)2D3 administration in moderate renal failure: A prospective double-blind trial

Table 3. Histomorphometric parameters of bone structure in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group + Vitamin D 400 U		Placebo group Vitamin D 400 U only		
	Before	After	Before	After	Normals[a]
Cancellous bone mass %	18.5 ± 0.9	17.1 ± 0.6	18.6 ± 1.9	21.8 ± 2.0	18.3 ± 0.7
Mean trabecular diameter *μm*	262 ± 10	270 ± 12	253 ± 9	280 ± 3	254 ± 4
Mean trabecular plate density *#/mm*	1.83 ± 0.13	1.64 ± 0.09	1.86 ± 0.15	1.74 ± 0.14	1.83 ± 0.05
Mean wall thickness *μm*	50.4 ± 1.7	51.2 ± 2.2	55.9 ± 2.7	60.7 ± 4.6	55.5 ± 1.2
Stainable aluminum at the bone-osteoid interface %	0	0	0	0	0

Normal at baseline → No Changes

[a] Age and sex-matched normal controls

Table 5. Dynamic histomorphometric parameters of bone in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group		Placebo group Vitamin D 400 U only		
	Before	After	Before	After	Normals[a]
Mineralization rate (*μm/day*)	0.59 ± 0.05	0.48 ± 0.08	0.47 ± 0.07	0.64 ± 0.07	0.51 ± 0.02
Doubly labelled trabecular surfaces %	3.01 ± .81	1.72 ± .41	2.6 ± 1.07	5.8 ± 1.26	3.76 ± 0.68
Mineralization lag time *days*	17.7 ± 3.70	16.4 ± 2.43	21.8 ± 2.18	17.5 ± 2.49	18.9 ± 0.78
Osteon remodelling time *days*	148 ± 34	187 ± 42	221 ± 45	158 ± 31	169 ± 2.2

[a] Age- and sex-matched normal controls

Normal at baseline → Labelling going in opposite directions (NS)

Baker LRI et al: Kidney International 35:661-669 , 1989

Table 3. In all patients, cancellous bone mass, mean trabecular diameter, mean trabecular plate density and mean wall thickness were within the normal range at the beginning of the study and after 12 months of therapy. No significant changes were observed in these parameters.

Table 5. Mineral apposition rate was not significantly different from normal controls at baseline or after therapy in all patients. Fraction of trabecular surfaces exhibiting double or single tetracycline labels was not different from normals at baseline. There was a trend towards a decrease in labelling after 12 months of treatment with 1,25(OH)2D3 and a trend in the placebo group toward an increase in tetracycline labelling. Both trends did not achieve statistical significance.

1,25(OH)2D3 administration in moderate renal failure: A prospective double-blind trial

Table 4. Histomorphometric parameters of bone formation and resorption in patients with moderate renal failure before and 12 months after therapy with $1,25(OH)_2D_3$ or placebo

	$1,25(OH)_2D_3$ group + Vitamin D 400 U		Placebo group Vitamin D 400 U only		
	Before	After	Before	After	Normals[d]
Lamellar osteoid volume mm^3/cm^3	4.66 ± 1.07	2.21 ± 0.67[b,c]	6.27 ± 2.51	6.49 ± 1.66	4.05 ± 0.09
Lamellar osteoid surface %	15.6 ± 2.59	11.3 ± 3.10	19.6 ± 7.34	19.3 ± 4.72	15.4 ± 0.58
Mean osteoid seam thickness μm	9.47 ± 0.85	6.98 ± 0.44[b,c]	9.76 ± 0.90	10.70 ± 1.24	9.51 ± 0.18
Woven osteoid volume mm^3/cm^3	1.95 ± 0.70[a,b]	0.76 ± 0.60[a,c]	0.58 ± 0.16[a]	2.27 ± 0.65[a,c]	0
Woven osteoid surface %	4.20 ± 1.24[a]	2.06 ± 1.27[a,c]	1.56 ± 0.39[a]	5.04 ± 1.34[a,c]	0
Bone-osteoblast interface %	6.18 ± 0.82[a]	1.81 ± 0.28[b,c]	7.18 ± 2.45[a]	8.28 ± 1.84[a]	2.70 ± 0.20
Osteoblastic index #/100 mm boundary length	429 ± 58[a]	127 ± 17[b,c]	444 ± 144[a]	492 ± 118[a]	174 ± 11
	osteoblastic index: number of osteoblasts per 100 mm trabecular boundary length				
Bone-osteoclast interface %	1.16 ± 0.16[a]	0.72 ± 0.26	1.64 ± 0.35	1.47 ± 0.35	0.73 ± 0.05
Osteoclastic index #/100 mm boundary length	42 ± 7.3[a]	24 ± 7.2	54 ± 13[a]	43 ± 10.6[a]	17 ± 0.91

[a] Differences between experimental groups and controls ($P < 0.05$)
[b] Differences between patients treated with $1,25(OH)_2D_3$ and placebo group ($P < 0.05$)
[c] Differences between baseline and 12 months of treatment or placebo within same group ($P < 0.05$)
[d] Age- and sex-matched normal controls

Woven osteoid, osteoblast index, & osteoclast index were very high at baseline.
All improved w/ calcitriol; woven osteoid worsened w/ placebo

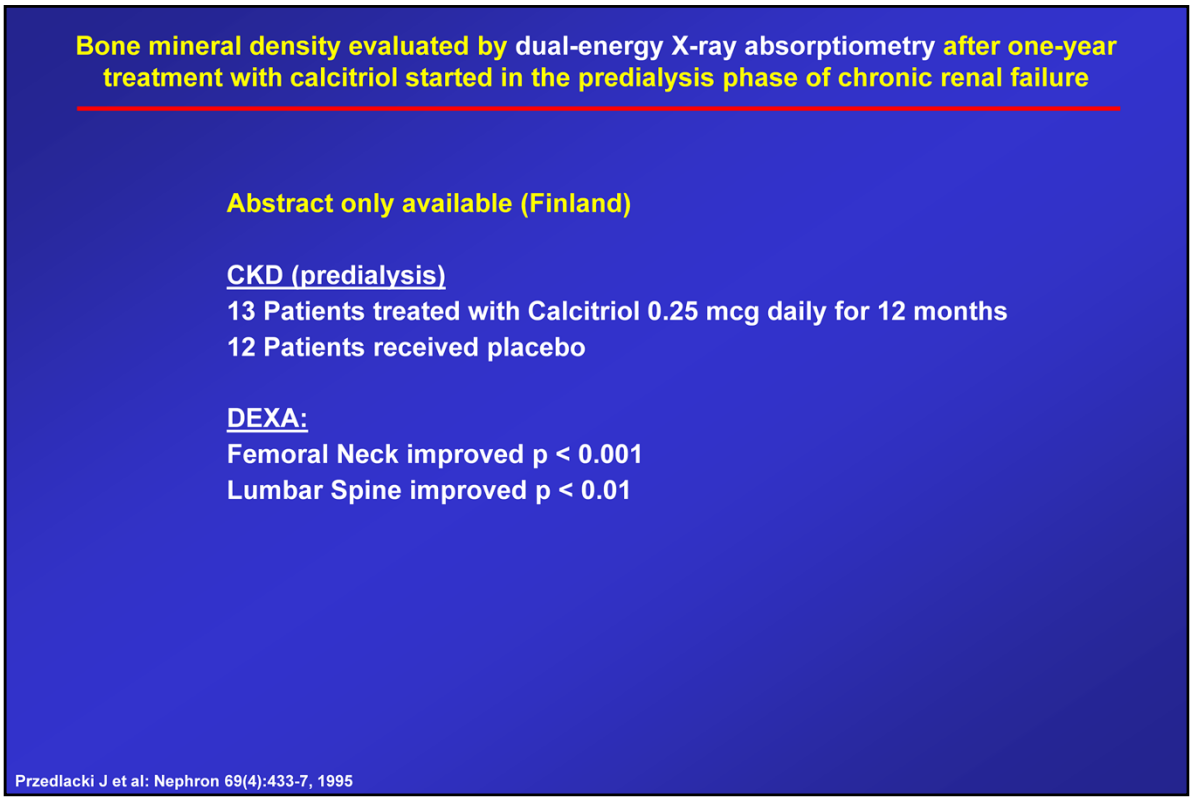
Baker LRI et al: Kidney International 35:661-669 , 1989

Volume of lamellar osteoid was above the normal range at baseline in two out of the seven patients who received 1,25(OH)2D and in two of the controls receiving placebo (Table 4). **Abnormal bone formation** as evidenced by the presence of **woven osteoid** was found at baseline in all biopsies. Mean thickness of lamellar osteoid seams was not increased. The number of osteoblasts per unit of trabecular boundary length and the bone-osteoblast interface were elevated at baseline in eight and nine patients, respectively; therefore, the mean values for both of these histo-morphometric parameters were significantly higher in the patients than in age- and sex-matched normal controls. Also, the number of osteoclasts per unit trabecular boundary length and the bone-osteoclast interface were elevated in nine and eleven patients, respectively, resulting in a significant increase in mean values of these parameters at baseline in both the patients that received placebo and 1,25(OH)1D3. Four patients exhibited trabecular fibrosis at baseline; those were the patients with the lowest creatinine clearances.

Administration of placebo for 12 months did not change lamellar osteoid volume and the number of osteoblasts and osteoclasts as well as bone-osteoblast interface and bone osteoclast interface were unchanged and continued to be above the normal range. Volume and surface of woven osteoid showed a further increase.

Administration of **1,25(OH)2D3** for 12 months resulted in a **significant decrease in**

lamellar osteoid volume and thickness. **Woven osteoid volume and surface were significantly reduced** and parameters of bone cells showed a decrease in the number of osteoblasts and osteoclasts, that is, the osteoblastic index and osteoclastic index. At the end of 1,25(OH)2D3 therapy five out of seven patients still exhibited some woven osteoid and the number of osteoblasts as well as bone-osteoblast interface was normal or low normal in all patients. The number of osteoclasts and bone-osteoclast interface was still above normal in three patients.



Thirteen patients in the predialysis phase of chronic renal failure (CRF) were treated with calcitriol (0.25 micrograms/day) and 12 with placebo. After 1 year of study, an increase in bone mineral density in the calcitriol group measured by dual-energy X-ray absorptiometry was seen for the femoral neck and lumbar spine when compared to the placebo group ($p < 0.001$ and $p < 0.01$, respectively). We conclude that a steady low dose of calcitriol started in the predialysis phase of CRF is beneficial to the patients with CRF. This may be partly due to suppression of secondary hyperparathyroidism.

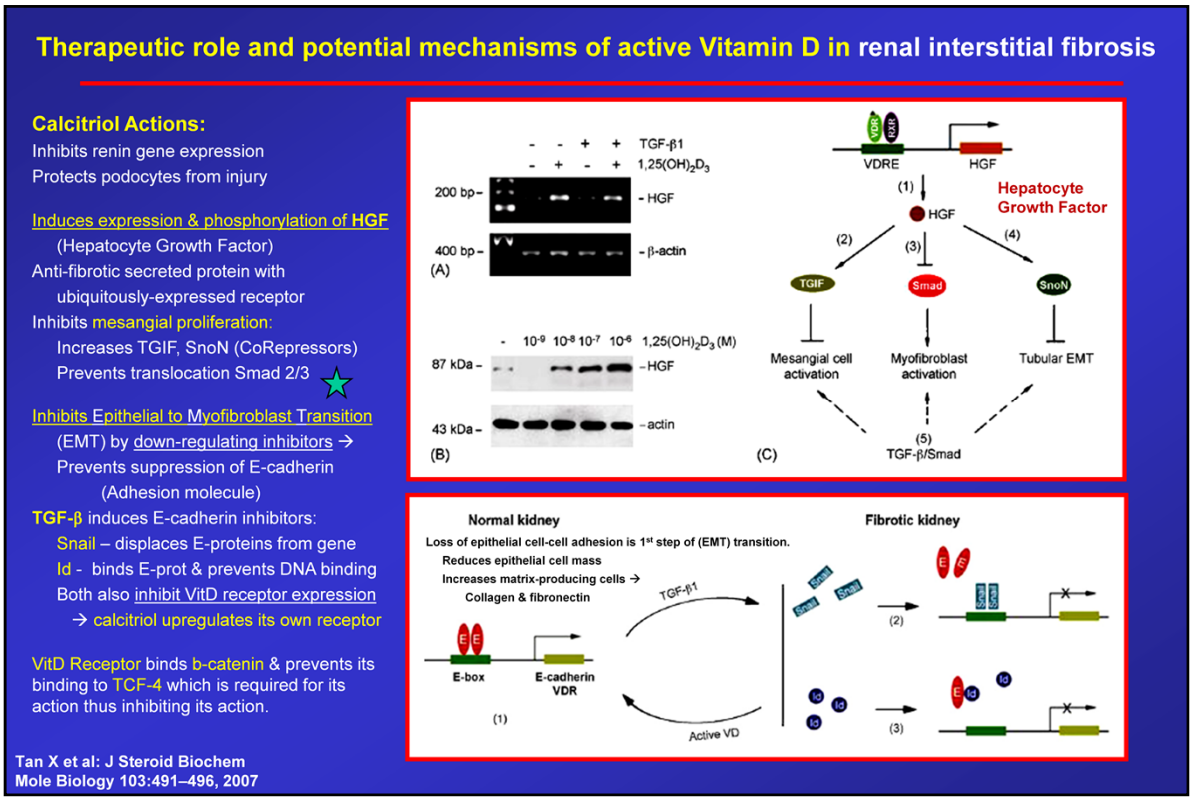


Fig. 1. Active Vitamin D may inhibit the activation of renal fibrogenic cells by inducing anti-fibrotic HGF expression. Active Vitamin D induces HGF mRNA expression and protein secretion in renal interstitial fibroblasts (NRK-49F), as shown by RT-PCR (A) and Western blot analyses (B). (C) Diagram shows the potential pathways leading to Vitamin D inhibition of renal fibrosis. Ligand-bound VDR *trans*-activate HGF gene expression (1). Increased HGF up-regulates Smad co-repressor TGIF expression and inhibits mesangial cell activation (2). HGF blocks the nuclear translocation of the activated-Smads in renal interstitial fibroblasts, thereby preventing myofibroblast activation (3). In tubular epithelial cells, HGF induces SnoN expression, thereby inhibiting TGF-/Smad-mediated tubular EMT (4). TGF-1, via its Smad signaling, promotes tubular EMT and myofibroblastic activation from glomerular mesangial cells and interstitial fibroblasts, respectively (5).

Fig. 2. Active Vitamin D preserves renal epithelial cell phenotypes by suppressing Snail and Id expression. In normal physiologic conditions, E-proteins, a family of bHLH transcription factors, bind to the E-boxes and *trans*-activate E-cadherin and Vitamin D receptor genes (1). In the fibrotic kidney, an increased Snail and Id proteins repress E-box-mediated gene expression through distinctive mechanisms. Snail displaces E-proteins from binding to the E-boxes via its DNA-binding capacity (2), whereas Id sequesters the gene activating activity of E-proteins through physically interacting with them (3).

Abstract Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D(3) or calcitriol, is essential in regulating a wide variety of biologic processes, such as calcium homeostasis, immune modulation, cell proliferation and differentiation. Clinical studies show that the circulating level of calcitriol is substantially reduced in patients with chronic renal insufficiency. Administration of **active Vitamin D** results in **significant amelioration of renal dysfunction and fibrotic lesions** in various experimental models of chronic kidney diseases. Active Vitamin D elicits its renal protective activity through multiple mechanisms, such as **inhibiting renal inflammation**, regulating **renin-angiotensin system** and **blocking mesangial cell activation**. Recent studies indicate that calcitriol induces **anti-fibrotic hepatocyte growth factor** expression, which in turn blocks the myofibroblastic activation and **matrix production** in interstitial fibroblasts. Furthermore, in vivo and in vitro studies demonstrate that active Vitamin D effectively blocks tubular epithelial to mesenchymal transition (EMT), a phenotypic conversion process that plays a central role in the evolution of renal interstitial fibrosis. Together, it is becoming increasingly clear that a **high level of active Vitamin D** may be obligatory in the maintenance of normal kidney structure and function. Thus, supplementation of active Vitamin D could be a rational strategy for the therapeutics of chronic kidney diseases.

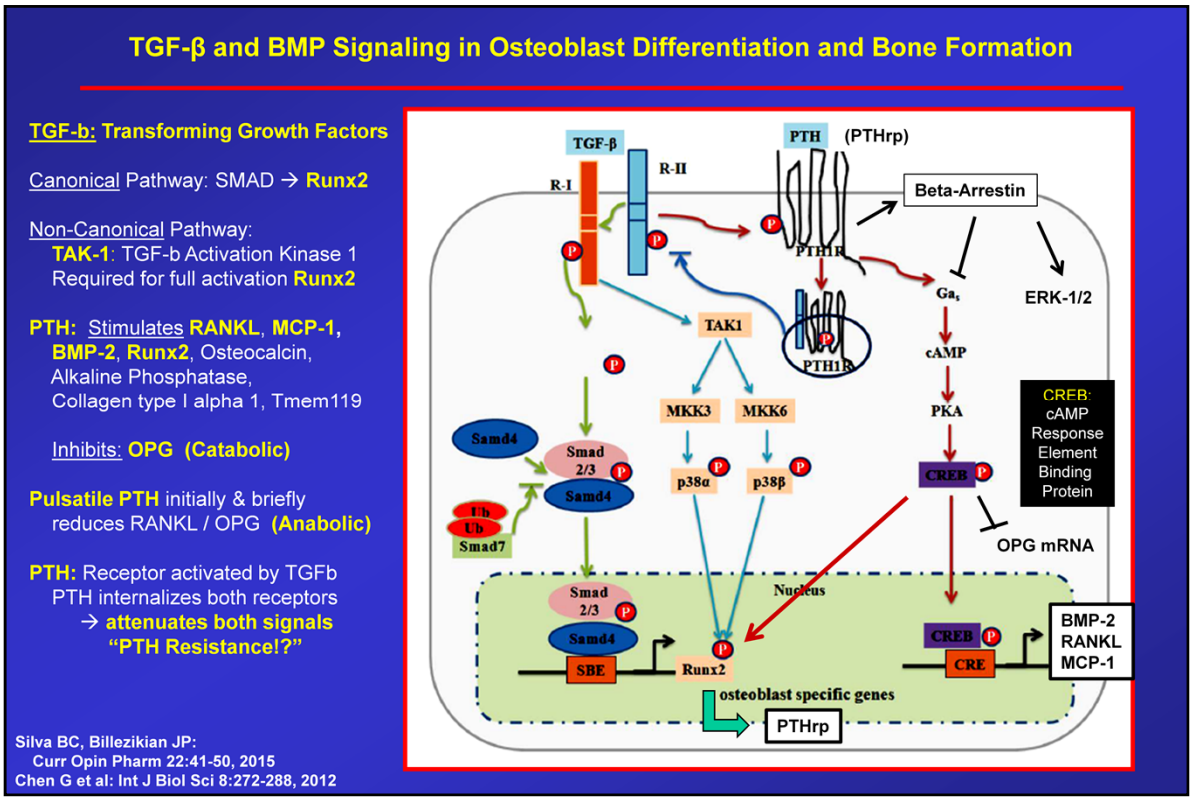


Figure 1. **TGF-β signaling and negative regulation in bone formation.**

Canonical Smad-dependent TGF-β signaling first binds to receptor type II (R-II) and receptor type I (R-I), and then signaling transduces to their Smads. Activated Smads form a complex with Smad4 and then translocate into the nucleus where they interact with other transcription factors to trigger target gene expression. Smad7 disrupts the activated Smad2/3 to form a complex with Smad4. The non-Smad-dependent TAK1 signaling pathway also regulates bone formation. PTH binding activates PTH1R to stimulate several downstream effectors. PTH binding also drives internalization of PTH1R-TGFβRII complex, which attenuates both TGF-β and PTH signaling on bone development. Transcriptional factor cAMP response element binding protein (CREB) mediates PTH signaling in osteoblasts.

Parathyroid hormone: anabolic and catabolic actions on the skeleton.

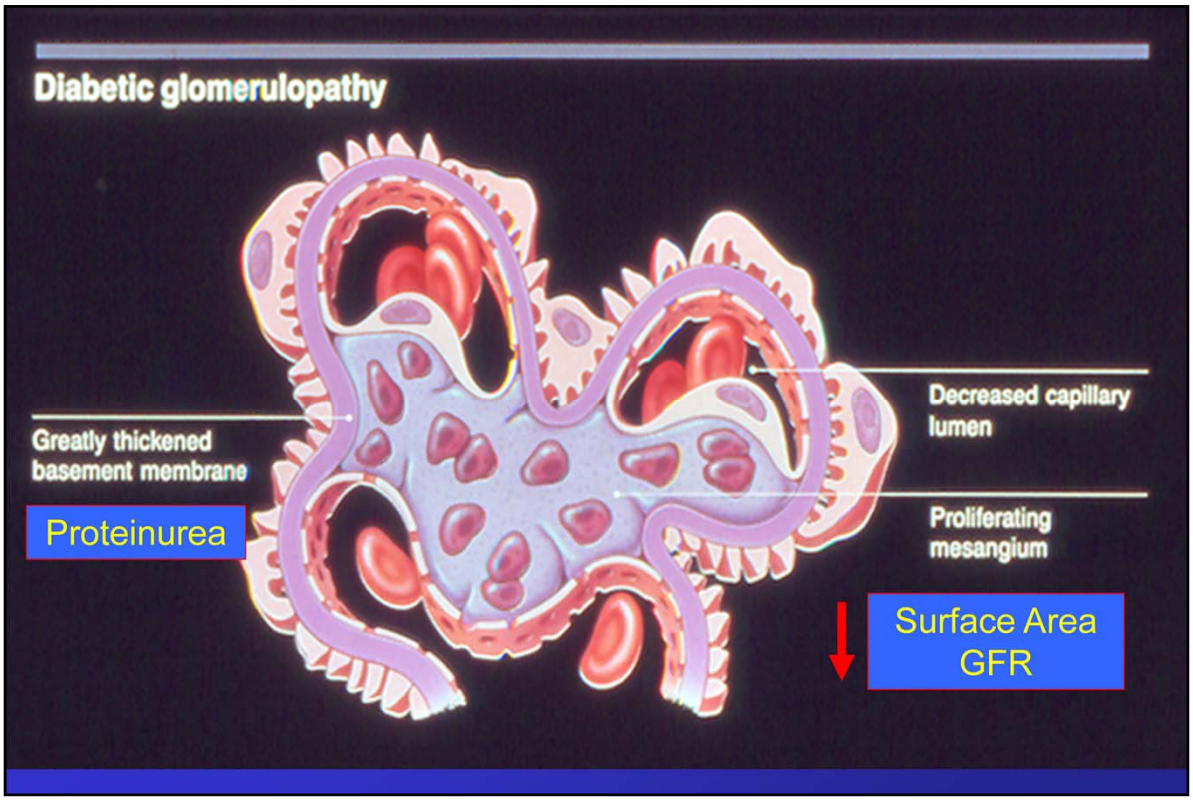
Parathyroid hormone (PTH) is essential for the maintenance of calcium homeostasis through, in part, its actions to regulate bone remodeling. While PTH stimulates both bone formation and bone resorption, the duration and periodicity of exposure to PTH governs the net effect on bone mass, that is whether it is catabolic or anabolic. PTH receptor signaling in osteoblasts and osteocytes can increase the RANKL/OPG ratio, increasing both osteoclast recruitment and osteoclast activity, and thereby stimulating bone resorption. In contrast, PTH-induced bone formation is explained, at least in part, by its ability to downregulate SOST/sclerostin

expression in osteocytes, permitting the anabolic Wnt signaling pathway to proceed. The two modes of administration of PTH, that is, continuous vs. intermittent, can regulate, in bone cells, different sets of genes; alternatively, the same sets of genes exposed to PTH in sustained vs. transient way, will favor bone resorption or bone formation, respectively. This article reviews the effects of PTH on bone cells that lead to these dual catabolic and anabolic actions on the skeleton.

TGF-β and BMP signaling in osteoblast differentiation and bone formation.

Transforming growth factor-beta (TGF-β)/bone morphogenic protein (BMP) signaling is involved in a vast majority of cellular processes and is fundamentally important throughout life. TGF-β/BMPs have widely recognized roles in bone formation during mammalian development and exhibit versatile regulatory functions in the body. Signaling transduction by TGF-β/BMPs is specifically through both canonical Smad-dependent pathways (TGF-β/BMP ligands, receptors and Smads) and non-canonical Smad-independent signaling pathway (e.g. p38 mitogen-activated protein kinase pathway, MAPK). Following TGF-β/BMP induction, both the Smad and p38 MAPK pathways converge at the Runx2 gene to control **mesenchymal precursor cell differentiation**. The coordinated activity of Runx2 and TGF-β/BMP-activated Smads is critical for formation of the skeleton. Recent advances in molecular and genetic studies using gene targeting in mice enable a better understanding of TGF-β/BMP signaling in bone and in the signaling networks underlying osteoblast differentiation and bone formation. This review summarizes the recent advances in our understanding of TGF-β/BMP signaling in bone from studies of genetic mouse models and human diseases caused by the disruption of TGF-β/BMP signaling. This review also highlights the different modes of cross-talk between TGF-β/BMP signaling and the signaling pathways of MAPK, Wnt, Hedgehog, Notch, and FGF in osteoblast differentiation and bone formation.

P: phosphorylation; Ub: ubiquitination.


Diabetic glomerulopathy
Greatly thickened basement membrane
Proteinurea
Decreased capillary lumen
Proliferating mesangium
Surface Area
GFR

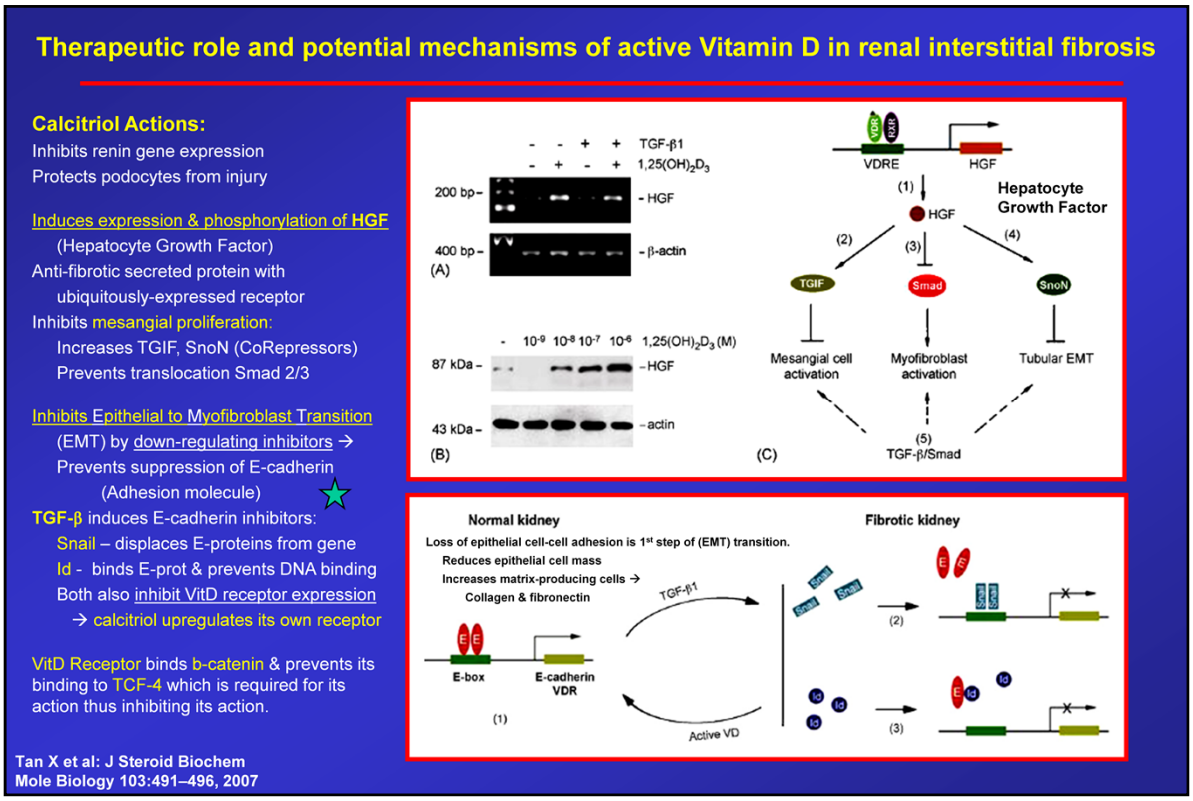


Fig. 1. Active Vitamin D may inhibit the activation of renal fibrogenic cells by inducing anti-fibrotic HGF expression. Active Vitamin D induces HGF mRNA expression and protein secretion in renal interstitial fibroblasts (NRK-49F), as shown by RT-PCR (A) and Western blot analyses (B). (C) Diagram shows the potential pathways leading to Vitamin D inhibition of renal fibrosis. Ligand-bound VDR *trans*-activate HGF gene expression (1). Increased HGF up-regulates Smad co-repressor TGIF expression and inhibits mesangial cell activation (2). HGF blocks the nuclear translocation of the activated-Smads in renal interstitial fibroblasts, thereby preventing myofibroblast activation (3). In tubular epithelial cells, HGF induces SnoN expression, thereby inhibiting TGF-/Smad-mediated tubular EMT (4). TGF-1, via its Smad signaling, promotes tubular EMT and myofibroblastic activation from glomerular mesangial cells and interstitial fibroblasts, respectively (5).

Fig. 2. Active Vitamin D preserves renal epithelial cell phenotypes by suppressing Snail and Id expression. In normal physiologic conditions, E-proteins, a family of bHLH transcription factors, bind to the E-boxes and *trans*-activate E-cadherin and Vitamin D receptor genes (1). In the fibrotic kidney, an increased Snail and Id proteins repress E-box-mediated gene expression through distinctive mechanisms. Snail displaces E-proteins from binding to the E-boxes via its DNA-binding capacity (2), whereas Id sequesters the gene activating activity of E-proteins through physically interacting with them (3).

Abstract

Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D(3) or calcitriol, is essential in regulating a wide variety of biologic processes, such as calcium homeostasis, immune modulation, cell proliferation and differentiation. Clinical studies show that the circulating level of calcitriol is substantially reduced in patients with chronic renal insufficiency. Administration of **active Vitamin D** results in **significant amelioration of renal dysfunction and fibrotic lesions** in various experimental models of chronic kidney diseases. Active Vitamin D elicits its renal protective activity through multiple mechanisms, such as **inhibiting renal inflammation**, regulating **renin-angiotensin system** and **blocking mesangial cell activation**. Recent studies indicate that calcitriol induces **anti-fibrotic hepatocyte growth factor** expression, which in turn blocks the myofibroblastic activation and **matrix production** in interstitial fibroblasts. Furthermore, in vivo and in vitro studies demonstrate that active Vitamin D effectively blocks tubular epithelial to mesenchymal transition (EMT), a phenotypic conversion process that plays a central role in the evolution of renal interstitial fibrosis. Together, it is becoming increasingly clear that a **high level of active Vitamin D** may be obligatory in the maintenance of normal kidney structure and function. Thus, supplementation of active Vitamin D could be a rational strategy for the therapeutics of chronic kidney diseases.

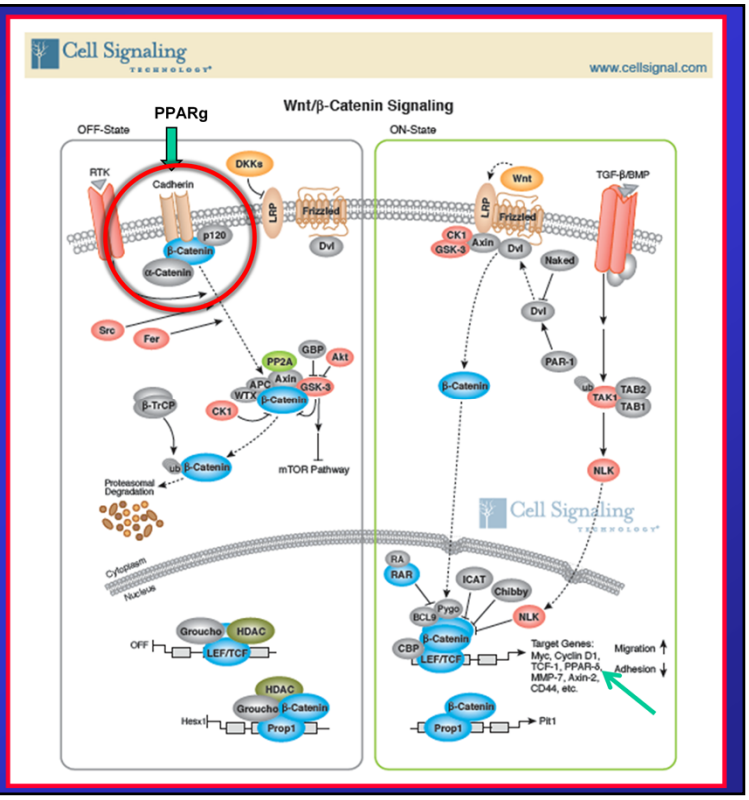
Cellular Control of Arterial Calcification

Wnt Pathway

Present in slime molds
Controls cell-cell communication
Embryonic Development
Maintains Adult Cell Differentiation
Cell Polarity

Wnt controls beta-Catenin
Wnt5a → PL-C → IP3, DAG
(increased in some cancers)

APC defic or b-Catenin mutation → excess stem cell renewal & proliferation



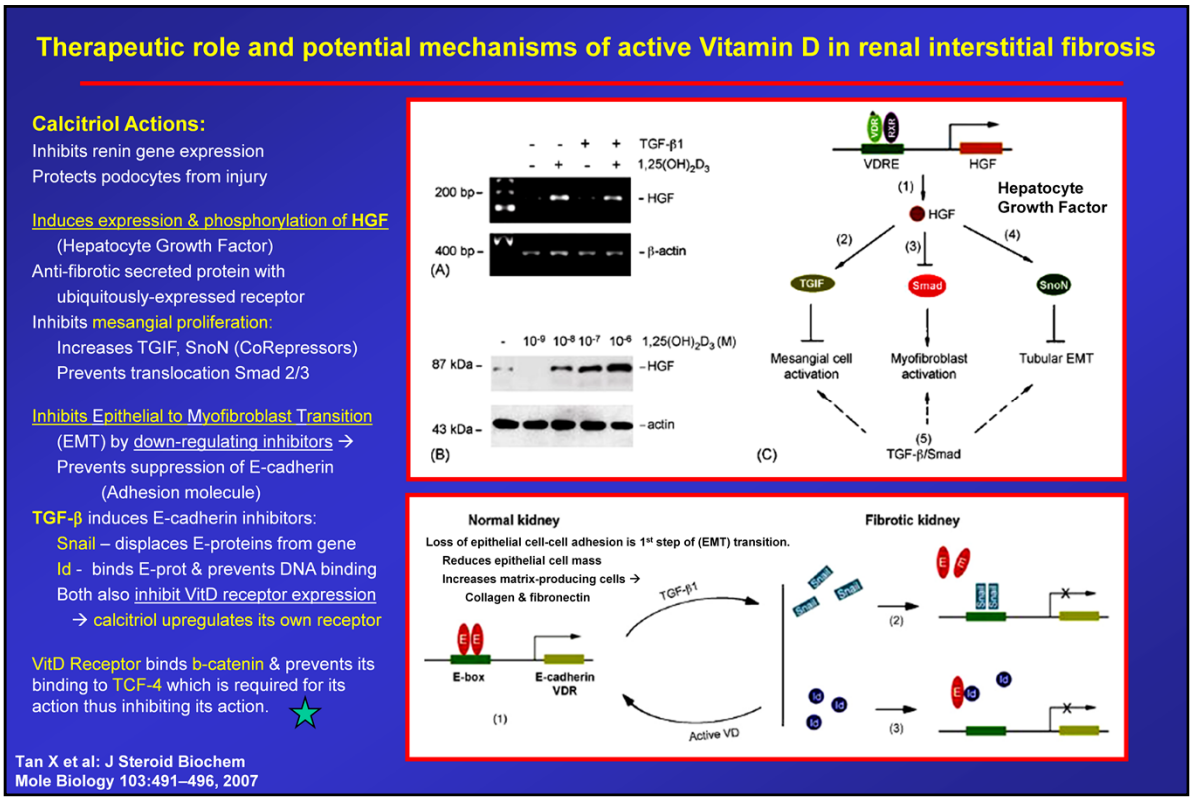


Fig. 1. Active Vitamin D may inhibit the activation of renal fibrogenic cells by inducing anti-fibrotic HGF expression. Active Vitamin D induces HGF mRNA expression and protein secretion in renal interstitial fibroblasts (NRK-49F), as shown by RT-PCR (A) and Western blot analyses (B). (C) Diagram shows the potential pathways leading to Vitamin D inhibition of renal fibrosis. Ligand-bound VDR *trans*-activate HGF gene expression (1). Increased HGF up-regulates Smad co-repressor TGIF expression and inhibits mesangial cell activation (2). HGF blocks the nuclear translocation of the activated-Smads in renal interstitial fibroblasts, thereby preventing myofibroblast activation (3). In tubular epithelial cells, HGF induces SnoN expression, thereby inhibiting TGF-/Smad-mediated tubular EMT (4). TGF-1, via its Smad signaling, promotes tubular EMT and myofibroblastic activation from glomerular mesangial cells and interstitial fibroblasts, respectively (5).

Fig. 2. Active Vitamin D preserves renal epithelial cell phenotypes by suppressing Snail and Id expression. In normal physiologic conditions, E-proteins, a family of bHLH transcription factors, bind to the E-boxes and *trans*-activate E-cadherin and Vitamin D receptor genes (1). In the fibrotic kidney, an increased Snail and Id proteins repress E-box-mediated gene expression through distinctive mechanisms. Snail displaces E-proteins from binding to the E-boxes via its DNA-binding capacity (2), whereas Id sequesters the gene activating activity of E-proteins through physically interacting with them (3).

Abstract Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D(3) or calcitriol, is essential in regulating a wide variety of biologic processes, such as calcium homeostasis, immune modulation, cell proliferation and differentiation. Clinical studies show that the circulating level of calcitriol is substantially reduced in patients with chronic renal insufficiency. Administration of **active Vitamin D** results in **significant amelioration of renal dysfunction and fibrotic lesions** in various experimental models of chronic kidney diseases. Active Vitamin D elicits its renal protective activity through multiple mechanisms, such as **inhibiting renal inflammation**, regulating **renin-angiotensin system** and **blocking mesangial cell activation**. Recent studies indicate that calcitriol induces **anti-fibrotic hepatocyte growth factor** expression, which in turn blocks the myofibroblastic activation and **matrix production** in interstitial fibroblasts. Furthermore, in vivo and in vitro studies demonstrate that active Vitamin D effectively blocks tubular epithelial to mesenchymal transition (EMT), a phenotypic conversion process that plays a central role in the evolution of renal interstitial fibrosis. Together, it is becoming increasingly clear that a **high level of active Vitamin D** may be obligatory in the maintenance of normal kidney structure and function. Thus, supplementation of active Vitamin D could be a rational strategy for the therapeutics of chronic kidney diseases.

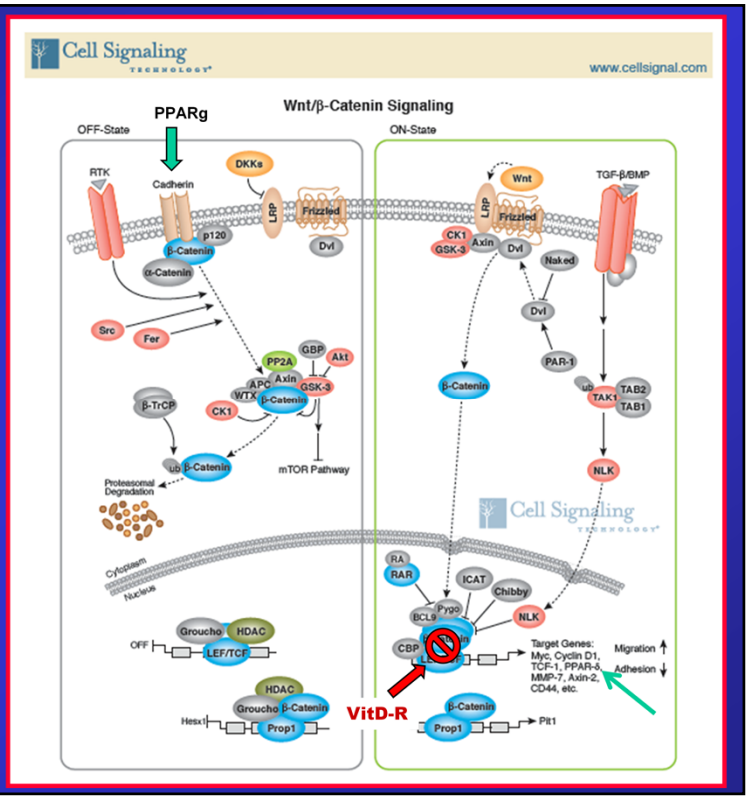
Cellular Control of Arterial Calcification

Wnt Pathway

Present in slime molds
Controls cell-cell communication
Embryonic Development
Maintains Adult Cell Differentiation
Cell Polarity

Wnt controls beta-Catenin
Wnt5a → PL-C → IP3, DAG
(increased in some cancers)

APC defic or b-Catenin mutation → excess stem cell renewal & proliferation



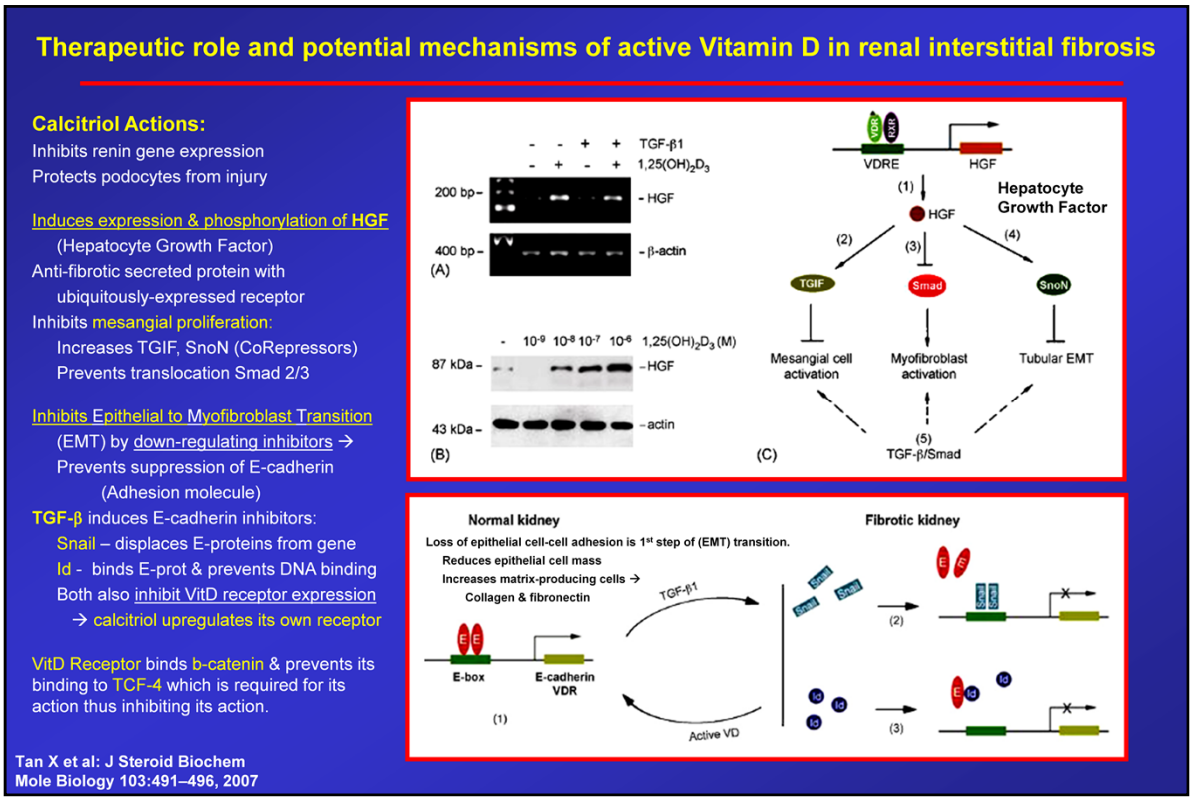
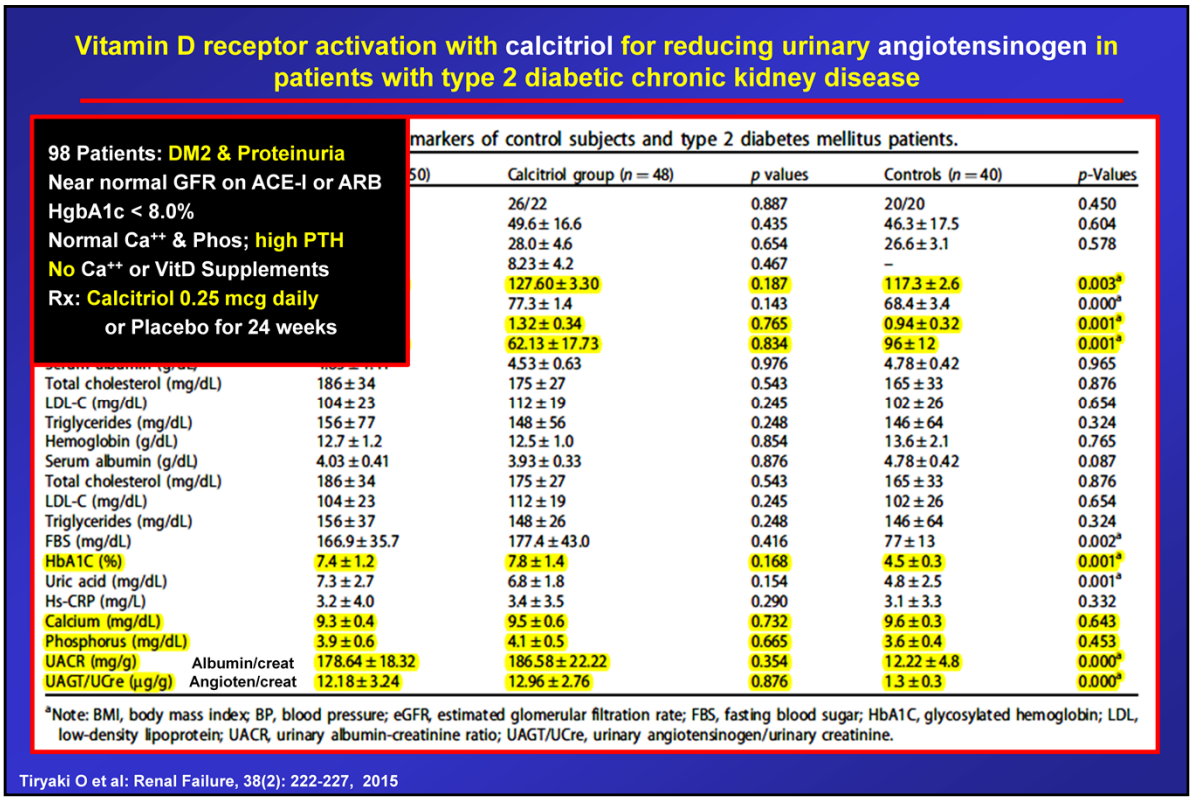


Fig. 1. Active Vitamin D may inhibit the activation of renal fibrogenic cells by inducing anti-fibrotic HGF expression. Active Vitamin D induces HGF mRNA expression and protein secretion in renal interstitial fibroblasts (NRK-49F), as shown by RT-PCR (A) and Western blot analyses (B). (C) Diagram shows the potential pathways leading to Vitamin D inhibition of renal fibrosis. Ligand-bound VDR *trans*-activate HGF gene expression (1). Increased HGF up-regulates Smad co-repressor TGIF expression and inhibits mesangial cell activation (2). HGF blocks the nuclear translocation of the activated-Smads in renal interstitial fibroblasts, thereby preventing myofibroblast activation (3). In tubular epithelial cells, HGF induces SnoN expression, thereby inhibiting TGF-/Smad-mediated tubular EMT (4). TGF-1, via its Smad signaling, promotes tubular EMT and myofibroblastic activation from glomerular mesangial cells and interstitial fibroblasts, respectively (5).

Fig. 2. Active Vitamin D preserves renal epithelial cell phenotypes by suppressing Snail and Id expression. In normal physiologic conditions, E-proteins, a family of bHLH transcription factors, bind to the E-boxes and *trans*-activate E-cadherin and Vitamin D receptor genes (1). In the fibrotic kidney, an increased Snail and Id proteins repress E-box-mediated gene expression through distinctive mechanisms. Snail displaces E-proteins from binding to the E-boxes via its DNA-binding capacity (2), whereas Id sequesters the gene activating activity of E-proteins through physically interacting with them (3).

Abstract Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D(3) or calcitriol, is essential in regulating a wide variety of biologic processes, such as calcium homeostasis, immune modulation, cell proliferation and differentiation. Clinical studies show that the circulating level of calcitriol is substantially reduced in patients with chronic renal insufficiency. Administration of **active Vitamin D** results in **significant amelioration of renal dysfunction and fibrotic lesions** in various experimental models of chronic kidney diseases. Active Vitamin D elicits its renal protective activity through multiple mechanisms, such as **inhibiting renal inflammation**, regulating **renin-angiotensin system** and **blocking mesangial cell activation**. Recent studies indicate that calcitriol induces **anti-fibrotic hepatocyte growth factor** expression, which in turn blocks the myofibroblastic activation and **matrix production** in interstitial fibroblasts. Furthermore, in vivo and in vitro studies demonstrate that active Vitamin D effectively blocks tubular epithelial to mesenchymal transition (EMT), a phenotypic conversion process that plays a central role in the evolution of renal interstitial fibrosis. Together, it is becoming increasingly clear that a **high level of active Vitamin D** may be obligatory in the maintenance of normal kidney structure and function. Thus, supplementation of active Vitamin D could be a rational strategy for the therapeutics of chronic kidney diseases.



Vitamin D receptor activation with calcitriol for reducing urinary angiotensinogen in patients with type 2 diabetic chronic kidney disease

98 Patients: DM2 & Proteinuria
Near normal GFR on ACE-I or ARB
HgbA1c < 8.0%
Normal Ca^{++} & Phos; high PTH
No Ca^{++} or VitD Supplements
Rx: Calcitriol 0.25 mcg daily
or Placebo for 24 weeks

markers of control subjects and type 2 diabetes mellitus patients.

		50)	Calcitriol group ($n = 48$)	p values	Controls ($n = 40$)	p-Values
			26/22	0.887	20/20	0.450
			49.6 ± 16.6	0.435	46.3 ± 17.5	0.604
			28.0 ± 4.6	0.654	26.6 ± 3.1	0.578
			8.23 ± 4.2	0.467	–	
			127.60 ± 3.30	0.187	117.3 ± 2.6	0.003[a]
			77.3 ± 1.4	0.143	68.4 ± 3.4	0.000[a]
			1.32 ± 0.34	0.765	0.94 ± 0.32	0.001[a]
			62.13 ± 17.73	0.834	96 ± 12	0.001[a]
			4.53 ± 0.63	0.976	4.78 ± 0.42	0.965
Total cholesterol (mg/dL)		186 ± 34	175 ± 27	0.543	165 ± 33	0.876
LDL-C (mg/dL)		104 ± 23	112 ± 19	0.245	102 ± 26	0.654
Triglycerides (mg/dL)		156 ± 77	148 ± 56	0.248	146 ± 64	0.324
Hemoglobin (g/dL)		12.7 ± 1.2	12.5 ± 1.0	0.854	13.6 ± 2.1	0.765
Serum albumin (g/dL)		4.03 ± 0.41	3.93 ± 0.33	0.876	4.78 ± 0.42	0.087
Total cholesterol (mg/dL)		186 ± 34	175 ± 27	0.543	165 ± 33	0.876
LDL-C (mg/dL)		104 ± 23	112 ± 19	0.245	102 ± 26	0.654
Triglycerides (mg/dL)		156 ± 37	148 ± 26	0.248	146 ± 64	0.324
FBS (mg/dL)		166.9 ± 35.7	177.4 ± 43.0	0.416	77 ± 13	0.002[a]
HbA1C (%)		7.4 ± 1.2	7.8 ± 1.4	0.168	4.5 ± 0.3	0.001[a]
Uric acid (mg/dL)		7.3 ± 2.7	6.8 ± 1.8	0.154	4.8 ± 2.5	0.001[a]
Hs-CRP (mg/L)		3.2 ± 4.0	3.4 ± 3.5	0.290	3.1 ± 3.3	0.332
Calcium (mg/dL)		9.3 ± 0.4	9.5 ± 0.6	0.732	9.6 ± 0.3	0.643
Phosphorus (mg/dL)		3.9 ± 0.6	4.1 ± 0.5	0.665	3.6 ± 0.4	0.453
UACR (mg/g)	Albumin/creat	178.64 ± 18.32	186.58 ± 22.22	0.354	12.22 ± 4.8	0.000[a]
UAGT/UCre (μg/g)	Angioten/creat	12.18 ± 3.24	12.96 ± 2.76	0.876	1.3 ± 0.3	0.000[a]

[a]Note: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1C, glycosylated hemoglobin; LDL, low-density lipoprotein; UACR, urinary albumin-creatinine ratio; UAGT/UCre, urinary angiotensinogen/urinary creatinine.

Tiryaki O et al: Renal Failure, 38(2): 222-227, 2015

Abstract

BACKGROUND: Recently, it has been reported that urinary angiotensinogen levels is a specific index of the intrarenal renin-angiotensin-aldosterone system (RAAS) status and it is significantly correlated with urinary albumin:creatinine (Cr) ratio in hypertensive patients. The aim of the present study was to assess the effect of activation of the Vitamin D receptor with calcitriol on albuminuria and urinary angiotensinogen as a novel biomarker of the intra-renal RAAS status in patients with diabetic nephropathy (DN).

METHODS: Ninety-eight patients with type 2 diabetes and albuminuria who were treated with RAAS inhibitors (angiotensin-converting enzyme inhibitor (ACE-i) or angiotensin receptor blocker (ARB)) have participated in this study. Patients were randomized to receive either placebo (n = 50) or 0.25 μg/day calcitriol (n = 48). We have examined urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio before and 24 weeks later after treatment in both group.

RESULTS: The mean urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio were significantly higher in patients with DN than in normal controls ($p < 0.001$). Urinary angiotensinogen:Cr ratio was significantly, positively correlated with urinary albumin:Cr ratio in both groups (in the placebo group; $p = 0.01$, $r = 0.4236$, in calcitriol group; $p = 0.01$, $r = 0.4564$).

CONCLUSION: These data indicated that administration of Vitamin D receptor activator in combination with RAAS inhibitors had an additional benefit in lowering albuminuria in patients with DN. More pronounced reduction of urinary albumin:Cr ratio that was positively correlated with angiotensinogen:Cr ratio in calcitriol group suggested that Vitamin D receptor activation might blunt albuminuria by reducing urinary angiotensinogen levels reflecting intra-renal RAAS status.

Vitamin D receptor activation with calcitriol for reducing urinary angiotensinogen in patients with type 2 diabetic chronic kidney disease

Table 1. Clinical characteristics and urinary markers of control subjects and type 2 diabetes mellitus patients.

Variables		Placebo ($n = 50$)	Calcitriol group ($n = 48$)	p values	Controls ($n = 40$)	p-Values
Sex, male/female		28/22	26/22	0.887	20/20	0.450
Age (years)		52.4 ± 18.6	49.6 ± 16.6	0.435	46.3 ± 17.5	0.604
BMI (kg/m^2)		27.6 ± 2.9	28.0 ± 4.6	0.654	26.6 ± 3.1	0.578
Duration of diabetes (years)		9.48 ± 5.9	8.23 ± 4.2	0.467	–	
Systolic BP (mmHg)		128.59 ± 4.92	127.60 ± 3.30	0.187	117.3 ± 2.6	0.003[a]
Diastolic BP (mmHg)		78.3 ± 2.0	77.3 ± 1.4	0.143	68.4 ± 3.4	0.000[a]
Serum creatinine (mg/dL)		1.24 ± 0.42	1.32 ± 0.34	0.765	0.94 ± 0.32	0.001[a]
eGFR (mL/min)		66.79 ± 16.13	62.13 ± 17.73	0.834	96 ± 12	0.001[a]
Serum albumin (g/dL)		4.83 ± 1.41	4.53 ± 0.63	0.976	4.78 ± 0.42	0.965
Total cholesterol (mg/dL)		186 ± 34	175 ± 27	0.543	165 ± 33	0.876
LDL-C (mg/dL)		104 ± 23	112 ± 19	0.245	102 ± 26	0.654
Triglycerides (mg/dL)		156 ± 77	148 ± 56	0.248	146 ± 64	0.324
Hemoglobin (g/dL)		12.7 ± 1.2	12.5 ± 1.0	0.854	13.6 ± 2.1	0.765
Serum albumin (g/dL)		4.03 ± 0.41	3.93 ± 0.33	0.876	4.78 ± 0.42	0.087
Total cholesterol (mg/dL)		186 ± 34	175 ± 27	0.543	165 ± 33	0.876
LDL-C (mg/dL)		104 ± 23	112 ± 19	0.245	102 ± 26	0.654
Triglycerides (mg/dL)		156 ± 37	148 ± 26	0.248	146 ± 64	0.324
FBS (mg/dL)		166.9 ± 35.7	177.4 ± 43.0	0.416	77 ± 13	0.002[a]
HbA1C (%)		7.4 ± 1.2	7.8 ± 1.4	0.168	4.5 ± 0.3	0.001[a]
Uric acid (mg/dL)		7.3 ± 2.7	6.8 ± 1.8	0.154	4.8 ± 2.5	0.001[a]
Hs-CRP (mg/L)		3.2 ± 4.0	3.4 ± 3.5	0.290	3.1 ± 3.3	0.332
Calcium (mg/dL)		9.3 ± 0.4	9.5 ± 0.6	0.732	9.6 ± 0.3	0.643
Phosphorus (mg/dL)		3.9 ± 0.6	4.1 ± 0.5	0.665	3.6 ± 0.4	0.453
UACR (mg/g)	Albumin/creat	178.64 ± 18.32	186.58 ± 22.22	0.354	12.22 ± 4.8	0.000[a]
UAGT/UCre (μg/g)	Angioten/creat	12.18 ± 3.24	12.96 ± 2.76	0.876	1.3 ± 0.3	0.000[a]

[a]Note: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; HbA1C, glycosylated hemoglobin; LDL, low-density lipoprotein; UACR, urinary albumin-creatinine ratio; UAGT/UCre, urinary angiotensinogen/urinary creatinine.

Tiryaki O et al: Renal Failure, 38(2): 222-227, 2015

Abstract

BACKGROUND: Recently, it has been reported that urinary angiotensinogen levels is a specific index of the intrarenal renin-angiotensin-aldosterone system (RAAS) status and it is significantly correlated with urinary albumin:creatinine (Cr) ratio in hypertensive patients. The aim of the present study was to assess the effect of activation of the Vitamin D receptor with calcitriol on albuminuria and urinary angiotensinogen as a novel biomarker of the intra-renal RAAS status in patients with diabetic nephropathy (DN).

METHODS: Ninety-eight patients with type 2 diabetes and albuminuria who were treated with RAAS inhibitors (angiotensin-converting enzyme inhibitor (ACE-i) or angiotensin receptor blocker (ARB)) have participated in this study. Patients were randomized to receive either placebo (n = 50) or 0.25 μg/day calcitriol (n = 48). We have examined urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio before and 24 weeks later after treatment in both group.

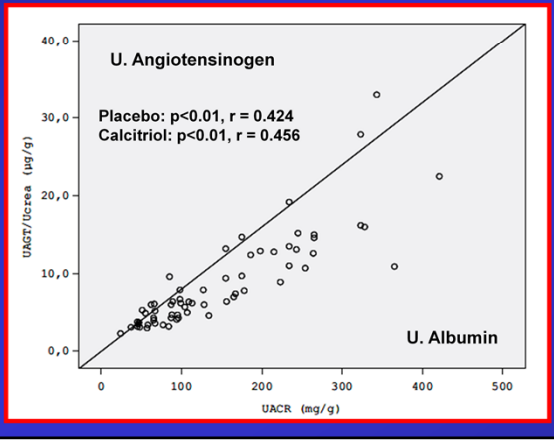
RESULTS: The mean urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio were significantly higher in patients with DN than in normal controls ($p < 0.001$). Urinary angiotensinogen:Cr ratio was significantly, positively correlated with urinary albumin:Cr ratio in both groups (in the placebo group; $p = 0.01$, $r = 0.4236$, in calcitriol group; $p = 0.01$, $r = 0.4564$).

CONCLUSION: These data indicated that administration of Vitamin D receptor activator in combination with RAAS inhibitors had an additional benefit in lowering albuminuria in patients with DN. More pronounced reduction of urinary albumin:Cr ratio that was positively correlated with angiotensinogen:Cr ratio in calcitriol group suggested that Vitamin D receptor activation might blunt albuminuria by reducing urinary angiotensinogen levels reflecting intra-renal RAAS status.

Vitamin D receptor activation with calcitriol for reducing urinary angiotensinogen in patients with type 2 diabetic chronic kidney disease

Table 3. Comparison of parameters in diabetic patients with plasma PTH level, UACR, eGFR, and UAGT/UCre before and after treatment with calcitriol and placebo.

Parameters		Placebo group ($n=50$) Baseline	Placebo group ($n=50$) 24 weeks later	p-Values	Calcitriol group ($n=48$) Baseline	Calcitriol group ($n=48$) 24 weeks later	p-Values
Plasma PTH level (ng/L)		126 ± 36	132 ± 42	0.869	128 ± 56	92 ± 28*	0.003
UACR (mg/g)	Albumin/creat	178.64 ± 18.32	161.28 ± 16.24&	0.09	186.58 ± 22.22	142.72 ± 12.38*	0.014
UAGT/UCre (μg/g)	Angioten/creat	12.18 ± 3.24	11.92 ± 2.64&	0.578	12.96 ± 2.76	8.64 ± 2.24*	0.012
eGFR (mL/min)		66.79 ± 16.13	63.54 ± 15.45&	0.776	62.13 ± 17.73	67.48 ± 14.33	0.009



98 Patients: DM2 & Proteinuria
Near normal GFR on ACE-I or ARB
HgbA1c < 8.0%
Normal Ca++ & Phos; high PTH
No Ca^{++} or VitD Supplements
Rx: Calcitriol 0.25 mcg daily
or Placebo for 24 weeks

GFR, PTH, Albuminuria,
& urinary Angiotensinogen
improved w/ calcitriol (0.25 mcg) therapy added to
RAAS inhibitor in diabetic nephropathy

Tiryaki O et al: Renal Failure, 38(2): 222-227, 2015

Background: Recently, it has been reported that urinary angiotensinogen levels is a specific index of the intrarenal renin-angiotensin-aldosterone system (RAAS) status and it is significantly correlated with urinary albumin:creatinine (Cr) ratio in hypertensive patients. The aim of the present study was to assess the effect of activation of the Vitamin D receptor with calcitriol on albuminuria and urinary angiotensinogen as a novel biomarker of the intra-renal RAAS status in patients with diabetic nephropathy (DN).

Methods: Ninety-eight patients with type 2 diabetes and albuminuria who were treated with RAAS inhibitors (angiotensin-converting enzyme inhibitor (ACE-i) or angiotensin receptor blocker (ARB)) have participated in this study. Patients were randomized to receive either placebo (n = 50) or 0.25 μg/day calcitriol (n = 48). We have examined urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio before and 24 weeks later after treatment in both group.

Results: The mean urinary albumin:Cr ratio and urinary angiotensinogen:Cr ratio were significantly higher in patients with DN than in normal controls ($p < 0.001$). Urinary angiotensinogen:Cr ratio was significantly, positively correlated with urinary albumin:Cr ratio in both groups (in the placebo group; $p = 0.01$, $r = 0.4236$, in calcitriol group; $p = 0.01$, $r = 0.4564$).

Conclusion: These data indicated that administration of Vitamin D receptor activator in combination with RAAS inhibitors had an additional benefit in lowering albuminuria in patients with DN. More pronounced reduction of urinary albumin:Cr ratio that was positively correlated with angiotensinogen:Cr ratio in calcitriol group suggested that Vitamin D receptor activation might blunt albuminuria by reducing urinary angiotensinogen levels reflecting intra-renal RAAS status.

A double-blind, randomized, placebo controlled trial of combined calcitriol and ergocalciferol versus ergocalciferol alone in chronic kidney disease with proteinuria

GFR 15-60, >1 gm Prot, 25VitD <30
VitD 40,000 U/week
Calcitriol 0.5 mcg twice **weekly** (n=36) or Placebo (n=32)
Age ~63 DM ~50% Prot ~3.5 gm PTH ~86 25VitD ~16

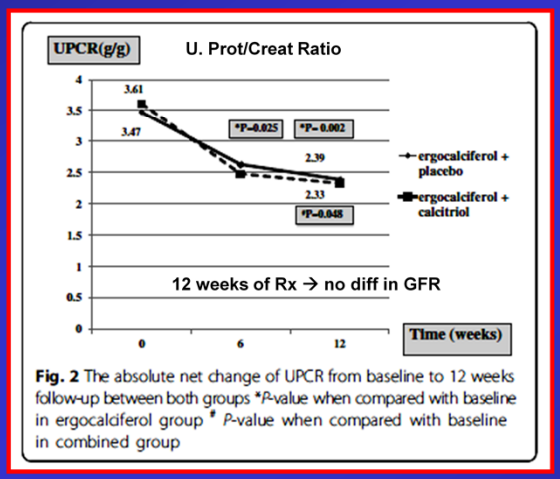
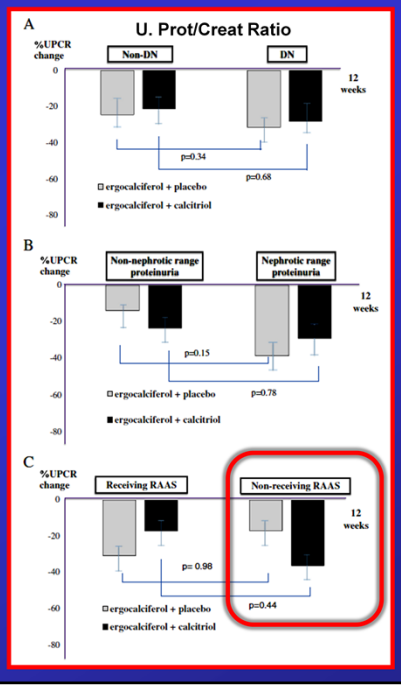


Fig. 2 The absolute net change of UPCR from baseline to 12 weeks follow-up between both groups *P-value when compared with baseline in ergocalciferol group # P-value when compared with baseline in combined group



Susantitaphong et al: BMC Nephrology 18:19, 2017

Background: KDOQI guideline suggests that nutritional vitamin D should be supplemented in chronic kidney disease (CKD) patients who have vitamin D insufficiency/deficiency. However, there are scarce data regarding the additional benefit of active vitamin D supplement in CKD patients who were receiving nutritional vitamin D supplement. This study was conducted to explore the effect of adding active vitamin D to nutritional vitamin D supplement on proteinuria and kidney function in CKD with vitamin D insufficiency/deficiency.

Methods: This double-blind, randomized placebo-controlled trial was performed to answer the above question. Sixty-eight patients with CKD stage 3–4, urine protein to creatinine ratio (UPCR) > 1 g/g, and serum 25OH-D level < 30 ng/mL were enrolled. Patients were randomly assigned to receive 12-week treatment with oral ergocalciferol plus placebo (n = 36) or oral ergocalciferol plus calcitriol (n = 32).

Results: The mean baseline values of UPCR of both groups were comparable (3.6±3.8 g/g in combined group and 3.5 ± 3.0 g/g in ergocalciferol group). Following 12-week treatment, there were significant reductions in UPCR from baseline in both groups (2.3 ± 2.1 g/g in combined group and 2.4 ± 2.0 g/g in ergocalciferol group). The percentage reductions

in UPCR of both groups were not significantly different. The mean eGFR and blood pressure did not differ between baseline and 12-week follow-up and between both groups. No severe hypercalcemia or serious side effects were noted in both groups.

Conclusions: The proteinuria lowering effect of ergocalciferol in CKD patients with vitamin D deficiency was demonstrated. Additional calcitriol supplement did not have more effects on proteinuria.

Calcitriol Analog Supplementation

A Randomized Multicenter Trial of Paricalcitol versus Calcitriol for Secondary Hyperparathyroidism in Stages 3–4 CKD

Stage 3-4 CKD (n=110) & PTH >120 pg/ml; excluded if using VitD or derivatives; DM??
Randomized: Calcitriol 0.25 mcg/d ($28 for 90 days) or Paricalcitol 1 mcg/d ($108 for 90 days) → titrated
Goal: reduce PTH ~50% Primary Endpoint: episodes Ca^{++} >10.5 mg/dl

Table 1. Demographics and baseline data

Characteristic	Paricalcitol Group (*n*=54)	Calcitriol Group (*n*=56)
Age (yr)	66.6±13.2	64.7±12.6
Weight (kg)	92.4±21.7	97.6±28.4
BP (mmHg)		
Systolic	132.5±16.5	136.5±18.2
Diastolic	71.1±11.9	71.7±11.7
Race		
African American	33 (61)	41 (73)
Caucasian	18 (33)	14 (25)
Other	3 (6)	1 (2)
Creatinine (mg/dl)	2.49±0.72	2.63±0.83
eGFR (ml/min per 1.73 m^2)	27.8±9.3	27.0±9.2
cCa (mg/dl)	9.32±0.35	9.36±0.40
Phosphorus (mg/dl)	3.66±0.56	3.74±0.52
PTH (pg/ml)	176 (142, 221)	209 (158, 287)
Albumin (g/dl)	3.9±0.36	3.75±0.38
Alkaline phosphatase (U/L)	80 (65, 104)	77.5 (70.75, 94.5)
Urine phosphorus/creatinine ratio (mg/g)	418 (341, 562)	435 (322, 504)

Data are expressed as the mean ±SD, *n* (%), or median (interquartile range). cCa, albumin-corrected calcium; PTH, parathyroid hormone.

Coyne DW et al: Clin J Am Soc Nephrol 9:1620–1626, 2014

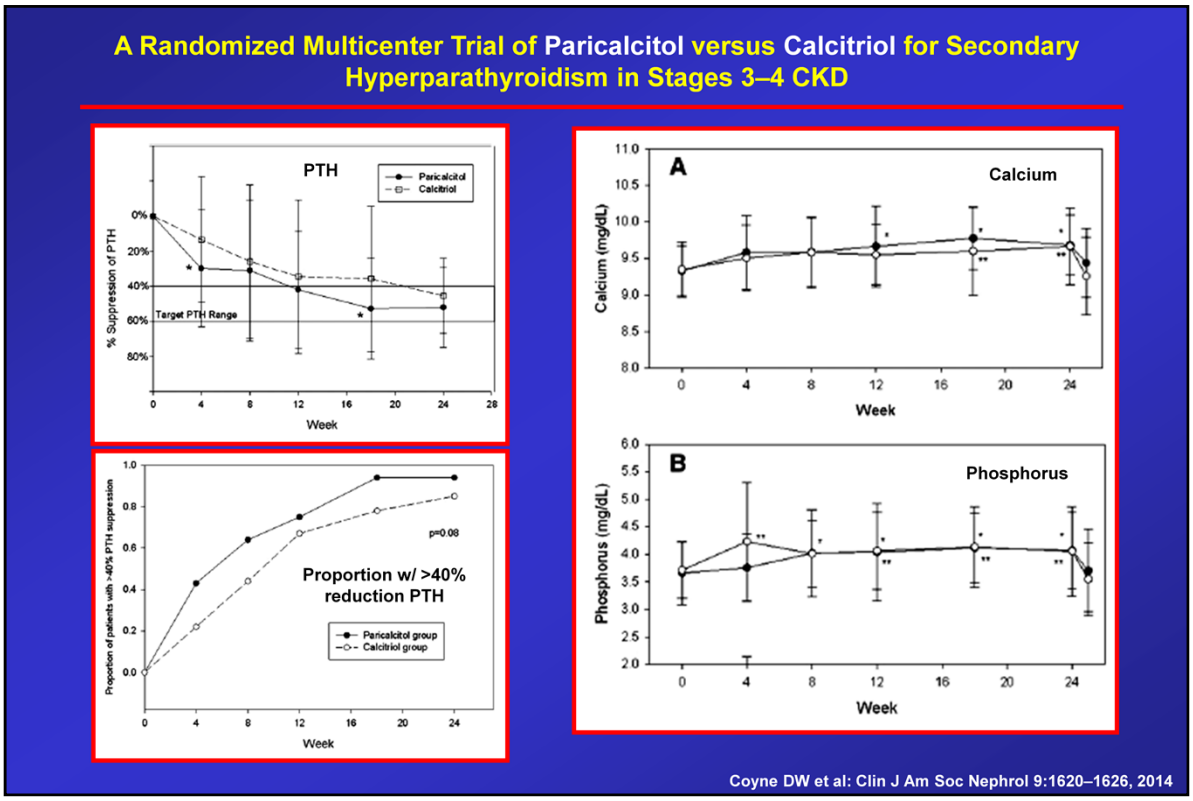
Background and objectives: Calcitriol is used to treat secondary hyperparathyroidism in patients with CKD. Paricalcitol is less calcemic and phosphatemic in preclinical studies and in some trials in dialysis patients, but head-to-head comparisons in non-dialysis patients are lacking. A large meta-analysis of trials concluded that these agents did not consistently reduce parathyroid hormone (PTH) and increased the risk of hypercalcemia and hyperphosphatemia. Therefore, the objective of this multicenter trial was to compare the rate of hypercalcemia between calcitriol and paricalcitol, while suppressing PTH 40%-60%.

Design, setting, participants, & measurements: Patients with stages 3–4 CKD (n=110) with a PTH level >120 pg/ml were recruited and randomized to 0.25 mg/d of calcitriol or 1 mg/d of paricalcitol between April 2009 and July 2011. Subsequent dose adjustments were by protocol to achieve 40%–60% PTH suppression below baseline. The primary endpoint was the rate of confirmed hypercalcemia of >10.5 mg/dl between groups.

Results: Forty-five patients in each group completed the 24 weeks of treatment. Both agents suppressed PTH effectively (-52% with paricalcitol and -46% with calcitriol; P=0.17), although the paricalcitol group reached a 40% reduction in PTH sooner at a median 8 weeks (interquartile range [IQR], 4, 12) versus 12 weeks (IQR, 8, 18; P=0.02) and had a lower pill burden of 240 (IQR, 180, 298) versus 292 (IQR,

231, 405; P=0.01). Confirmed hypercalcemia was very low in both groups (three with paricalcitol and one with calcitriol) and was not significantly different (P=0.36). Both groups had small increases in calcium and phosphorus levels (0.3–0.4 mg/dl in each electrolyte) and significant decreases in alkaline phosphatase, a marker of high bone turnover, with no significant differences between groups.

Conclusions: These results show that both calcitriol and paricalcitol achieved sustained PTH and alkaline phosphatase suppression in stages 3–4 CKD, with small effects on serum calcium and phosphorus and a low incidence of hypercalcemia.



Background and objectives: Calcitriol is used to treat secondary hyperparathyroidism in patients with CKD. Paricalcitol is less calcemic and phosphatemic in preclinical studies and in some trials in dialysis patients, but head-to-head comparisons in non-dialysis patients are lacking. A large meta-analysis of trials concluded that these agents did not consistently reduce parathyroid hormone (PTH) and increased the risk of hypercalcemia and hyperphosphatemia. Therefore, the objective of this multicenter trial was to compare the rate of hypercalcemia between calcitriol and paricalcitol, while suppressing PTH 40%-60%.

Design, setting, participants, & measurements: Patients with stages 3–4 CKD (n=110) with a PTH level >120 pg/ml were recruited and randomized to 0.25 mg/d of calcitriol or 1 mg/d of paricalcitol between April 2009 and July 2011. Subsequent dose adjustments were by protocol to achieve 40%–60% PTH suppression below baseline. The primary endpoint was the rate of confirmed hypercalcemia of >10.5 mg/dl between groups.

Results: Forty-five patients in each group completed the 24 weeks of treatment. Both agents suppressed PTH effectively (-52% with paricalcitol and -46% with calcitriol; P=0.17), although the paricalcitol group reached a 40% reduction in PTH sooner at a median 8 weeks (interquartile range [IQR], 4, 12) versus 12 weeks (IQR, 8, 18; P=0.02) and had a lower pill burden of 240 (IQR, 180, 298) versus 292 (IQR,

231, 405; P=0.01). Confirmed hypercalcemia was very low in both groups (three with paricalcitol and one with calcitriol) and was not significantly different (P=0.36). Both groups had small increases in calcium and phosphorus levels (0.3–0.4 mg/dl in each electrolyte) and significant decreases in alkaline phosphatase, a marker of high bone turnover, with no significant differences between groups.

Conclusions: These results show that both calcitriol and paricalcitol achieved sustained PTH and alkaline phosphatase suppression in stages 3–4 CKD, with small effects on serum calcium and phosphorus and a low incidence of hypercalcemia.

A Randomized Multicenter Trial of Paricalcitol versus Calcitriol for Secondary Hyperparathyroidism in Stages 3–4 CKD

Outcome	Paricalcitol Group (*n*=53)	Calcitriol Group (*n*=54)	*P* Value
Primary outcome			
Confirmed cCa>10.5 mg/dl	3 (5.7)	1 (1.9)	0.36
Secondary outcomes			
Any cCa>10.5 mg/dl	7 (13.2)	4 (7.4)	0.36
>40% PTH reduction	52 (98)	47 (87)	0.03
>60% PTH reduction	45 (83)	28 (52)	<0.001
Change in PTH, 24 wk (%)[a]	−52±23	−46±21	0.17
Total capsules	240 (180, 298)	292 (231, 405)	0.01
Change in cCa (mg/dl)[a]	+0.38 (0.10, 0.60)	+0.28 (0.14, 0.52)	0.27
Change in phosphorus (mg/dl)[a]	+0.2 (−0.1, 0.7)	+0.3 (0.0, 0.6)	0.88
Change in alkaline phosphatase (U/L)[a]	−9.0 (−22.2, 1.0)	−13.0 (−23.5, −8.0)	0.32
Any phosphorus>4.5 mg/dl	21 (40)	28 (52)	0.21
eGFR (ml/min per 1.73 m^2), 24 wk[a]	24.0±8.6	22.6±9.6	0.45
Urine phosphorus/creatinine ratio (mg/g), 24 wk	377 (292, 528)	414 (326, 514)	0.62

Adverse Event	Paricalcitol Group (*n*=53)	Calcitriol Group (*n*=54)
Any adverse event	37 (70)	35 (65)
Serious adverse event	11 (21)	14 (26)
Event type		
Dermatologic	7 (13)	7 (13)
Neurologic	11 (21)	6 (11)
Gastrointestinal	10 (19)	4 (7)
Genitourinary	7 (13)	5 (9)
Endocrine	4 (8)	10 (19)
Respiratory	7 (13)	7 (13)
Musculoskeletal	15 (28)	12 (22)
Cardiac	9 (17)	7 (13)
Psychiatric	2 (4)	2 (4)
Other	6 (12)	6 (11)

Mean final dosages:
Paricalcitol 1.3±0.8 mcg/d & Calcitriol 0.5±0.3 mcg/d

No substantial difference in endpoints or adverse events
4-fold difference in price

Can we assume that calcitriol will produce the same results as paricalcitol in clinical trials?

Most studies are now done with paricalcitrol

Coyne DW et al: Clin J Am Soc Nephrol 9:1620–1626, 2014

Background and objectives: Calcitriol is used to treat secondary hyperparathyroidism in patients with CKD. Paricalcitol is less calcemic and phosphatemic in preclinical studies and in some trials in dialysis patients, but head-to-head comparisons in non-dialysis patients are lacking. A large meta-analysis of trials concluded that these agents did not consistently reduce parathyroid hormone (PTH) and increased the risk of hypercalcemia and hyperphosphatemia. Therefore, the objective of this multicenter trial was to compare the rate of hypercalcemia between calcitriol and paricalcitol, while suppressing PTH 40%-60%.

Design, setting, participants, & measurements: Patients with stages 3–4 CKD (n=110) with a PTH level >120 pg/ml were recruited and randomized to 0.25 mg/d of calcitriol or 1 mg/d of paricalcitol between April 2009 and July 2011. Subsequent dose adjustments were by protocol to achieve 40%–60% PTH suppression below baseline. The primary endpoint was the rate of confirmed hypercalcemia of >10.5 mg/dl between groups.

Results: Forty-five patients in each group completed the 24 weeks of treatment. Both agents suppressed PTH effectively (-52% with paricalcitol and -46% with calcitriol; P=0.17), although the paricalcitol group reached a 40% reduction in PTH sooner at a median 8 weeks (interquartile range [IQR], 4, 12) versus 12 weeks (IQR, 8, 18; P=0.02) and had a lower pill burden of 240 (IQR, 180, 298) versus 292 (IQR,

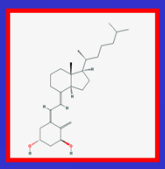
231, 405; P=0.01). Confirmed hypercalcemia was very low in both groups (three with paricalcitol and one with calcitriol) and was not significantly different (P=0.36). Both groups had small increases in calcium and phosphorus levels (0.3–0.4 mg/dl in each electrolyte) and significant decreases in alkaline phosphatase, a marker of high bone turnover, with no significant differences between groups.

Conclusions: These results show that both calcitriol and paricalcitol achieved sustained PTH and alkaline phosphatase suppression in stages 3–4 CKD, with small effects on serum calcium and phosphorus and a low incidence of hypercalcemia.

Effect of alfacalcidol on natural course of renal bone disease in mild to moderate renal failure

176 Patients in Europe CCr 15-50 cc/min
Normal Ca^{++}, Alk Phos, X-Rays
Some w/ Diabetes; 60% men; most had abnormal bone
No previous or current Vitamin D or Analogs

Alfacalcidiol: 0.25 mcg qAM → 1.00 mcg qOD
unless Ca^{++} high
Allowed up to 500 mg calcium supplement daily
Treated for 2 years



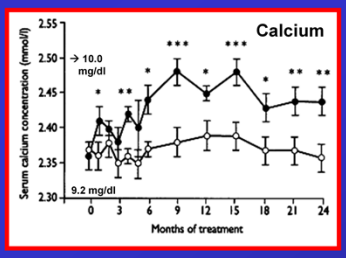


TABLE IV—*Changes in biochemical and radiographic features between start of study and after 6, 12, 18, and 24 months in 89 patients given alfacalcidol and 87 patients given placebo. Values are means (standard errors)*

	Months of treatment with alfacalcidol				Months of treatment with placebo				P values*	
	6	12	18	24	6	12	18	24	Treatment	Time
Serum concentrations:										
Creatinine (μmol/l)	32·3 (7·2)	57·6 (14·0)	59·3 (12·1)	78·8 (15·6)	26·8 (8·2)	39·6 (9·4)	44·2 (11·7)	74·1 (18·7)	0·41	<0·001
Corrected calcium (mmol/l)	0·08 (0·02)	0·08 (0·02)	0·07 (0·02)	0·07 (0·02) +0.3 mg/dL	0·00 (0·01)	−0·01 (0·02)	−0·01 (0·03)	−0·01 (0·03)	<0·001	0·91
Phosphate (mmol/l)	−0·04 (0·03)	−0·04 (0·03)	0·00 (0·03)	0·13 (0·05)	−0·04 (0·03)	−0·04 (0·03)	−0·06 (0·04)	−0·06 (0·06)	0·48	<0·001
Alkaline phosphatase (IU/l)	−25·5 (5·2)	−23·3 (6·1)	−18·4 (6·9)	−5·7 (6·8)	−8·1 (4·7)	0·8 (4·5)	12·6 (6·1)	19·8 (6·6)	<0·001	<0·001
Intact parathyroid hormone (pmol/l)	−2·9 (1·7)	−1·6 (0·9)	−1·6 (1·2)	0·6 (1·0) +6 pg/ml	2·0 (0·6)	5·9 (1·2)	7·3 (1·4)	8·1 (2·1) +76 pg/ml	<0·001	<0·001
Creatinine clearance (ml/min)	−1·3 (1·2)	−3·5 (1·4)	−3·5 (1·7)	−5·7 (1·0)	−3·1 (1·0)	−3·3 (1·4)	−2·8 (1·6)	−4·0 (2·0)	0·94	0·03
24 Hour urine excretion†:										
Creatinine (mmol/day)	−0·96 (07·1)	−1·28 (0·72)	−1·19 (0·62)	−1·87 (0·52)	−1·14 (0·55)	−0·42 (0·46)	−0·05 (0·79)	−0·82 (0·68)	0·36	0·06
Calcium (mmol/day)	0·47 (0·19)	0·80 (0·26)	0·70 (0·27)	0·87 (0·27)	0·36 (0·50)	−0·08 (0·24)	−0·07 (0·25)	0·56 (0·35)	0·06	0·72
Phosphate (mmol/day)	−2·74 (1·67)	−3·52 (1·73)	−5·46 (1·89)	−5·73 (1·48)	−2·69 (1·88)	−1·86 (1·51)	−1·98 (2·01)	−2·82 (1·76)	0·34	0·02
Hydroxyproline (μmol/day)	−93·7 (21·4)	−69·0 (21·6)	−84·4 (23·4)	−62·1 (23·4)	−57·7 (29·5)	−25·7 (27·7)	−27·3 (26·6)	10·7 (23·6)	0·09	0·84

Hamdy NAT et al: BMJ 310:358-363, 1995

Table IV: The group given alfacalcidol showed a small, nonsignificant decrease in serum concentrations of parathyroid hormone after six months of treatment and then a slow rise so that, at the end of two years, parathyroid hormone concentrations were the same as before treatment. In contrast, the group given placebo showed a significant, progressive, twofold increase in

concentrations of parathyroid hormone (table IV). The changes in serum activity of alkaline phosphatase were similar to those observed with serum parathyroid hormone concentrations in both groups. Thus, in patients given alfacalcidol, alkaline phosphatase activity decreased by 15% in the first six months of treatment, remained so for most of the duration of treatment, but had risen to pretreatment values by the end of the study. In the group given placebo, enzyme activity significantly and progressively increased and was significantly higher than in the alfacalcidol treated group at the end of treatment

Abstract

Objective - To determine whether alfacalcidol used in management of overt renal bone disease may safely prevent renal bone disease when used earlier in course of renal failure.

Design - Double blind, prospective, randomized, placebo controlled study.

Setting - 17 nephrology centers from Belgium, France, the Netherlands, and the United Kingdom.

Subjects - 176 patients aged 18-81 with mild to moderate chronic renal failure (creatinine clearance 15-50 ml/mi) and with no clinical, biochemical, or radiographic evidence of bone disease.

Interventions - Alfacalcidol 0.25 ug (titrated according to serum calcium concentration) or placebo given for two years.

Main outcome measures - Quantitative histology of bone to assess efficacy of treatment and renal function to assess safety.

Results - 132 patients had histological evidence of bone disease at start of study. Biochemical, radiographic, and histological indices of bone metabolism were similar for the 89 patients given alfacalcidol and the 87 controls given placebo. After treatment, mean serum alkaline phosphatase activity and intact parathyroid hormone concentration had increased by 13% and 126% respectively in controls but had not changed in patients given alfacalcidol ($P< 0.001$). Hypercalcemic episodes occurred in 10 patients given alfacalcidol (but responded to decreases in drug dose) and in three controls. Histological indices of bone turnover significantly improved in patients given alfacalcidol and significantly deteriorated in controls: among patients with abnormal bone histology before treatment, bone disease resolved in 23 (42%) of those given alfacalcidol compared with two (4%) of the controls ($P < 0.001$). There was no difference in rate of progression of renal failure between the two groups.

Conclusion - Early administration of alfacalcidol can safely and beneficially alter the natural course of renal bone disease in patients with mild to moderate renal failure.

Effect of alfacalcidol on natural course of renal bone disease in mild to moderate renal failure

TABLE V—*Semiquantitative and quantitative histological changes at the end of the study in 72 patients given alfacalcidol and 62 patients given placebo according to whether histological abnormalities were present at start of study. Values are means (standard errors)*

	Histological abnormalities at start of study			No histological abnormalities at start of study		
	Alfacalcidol (n=55)	Placebo (n=45)	P value†	Alfacalcidol (n=17)	Placebo (n=17)	P value†
Semiquantitative changes (% of patients affected):						
Degree of fibrosis	−0·58 (0·1)***	0·07 (0·1)	0·0002	0·53 (0·2)**	0·59 (0·2)**	0·88
Maximum No of osteoid lamellae	−0·73 (0·2)***	0·32 (0·2)	0·002	0·35 (0·5)	0·18 (0·3)	0·47
Quantitative changes‡:						
Bone volume (%)	1·22 (0·9)	1·09 (1·1)	0·75	0·29 (2·2)	0·83 (1·7)	0·9
Osteoid volume (%)	−0·30 (0·1)***	0·09 (0·1)	0·005	0·10 (0·1)	0·14 (0·1)	0·2
Osteoid surface (%)	−6·85 (1·8)**	1·35 (1·6)	0·008	0·44 (2·5)	0·80 (3·1)	0·2
Osteoblast surface (%)	−0·54 (0·3)**	0·37 (0·3)	0·009	0·58 (0·2)*	0·40 (0·2)	0·3
No of osteoblasts/mm² (%)	−0·38 (0·1)**	0·24 (0·2)	0·007	0·33 (0·1)*	0·20 (0·1)	0·26
Osteoid thickness	−0·49 (0·4)	0·05 (0·4)	0·37	−0·22 (0·4)	2·10 (0·6)**	0·004
Eroded surface (%)	−3·76 (1·1)***	0·45 (0·9)	0·04	−1·06 (1·9)	4·50 (2·3)	0·054
Active eroded surface (% of bone surface)	−0·86 (0·5)	0·49 (0·3)	0·0006	0·56 (0·4)	0·26 (0·4)	0·76
Osteoclast surface (%)	−0·30 (0·2)	0·17 (0·1)	0·002	0·16 (0·1)	0·03 (0·1)	0·51
No of osteoclasts/mm²	−0·07 (0·04)	0·05 (0·03)	0·001	0·04 (0·03)	0·01 (0·03)	0·76
Mineral apposition rate (mm/day)	−0·05 (0·04)	0·01 (0·04)	0·34	0·05 (0·1)	−0·03 (0·1)	0·53
Bone formation rate (mm³/mm³/day)	−4·66 (1·9)*	0·51 (1·7)	0·15	6·29 (4·5)	3·91 (2·4)	0·65
Mineralising surface (% of bone surface)	−1·99 (0·8)*	−0·15 (0·6)	0·16	2·35 (1·8)	1·60 (0·9)	0·59
Mineralising surface	2·68 (3·5)*	−3·41 (2·6)	0·26	14·55 (8·8)	−2·63 (8·9)	0·22
Mineralisation lag time (days)	−0·75 (0·8)*	−9·56 (0·8)	0·93	−2·55 (1·3)	2·07 (2·1)	0·05

* P<0·05, **P<0·01, ***P<0·001; differences within treatment groups.
† Differences between treatment groups.

Hamdy NAT et al: BMJ 310:358-363, 1995

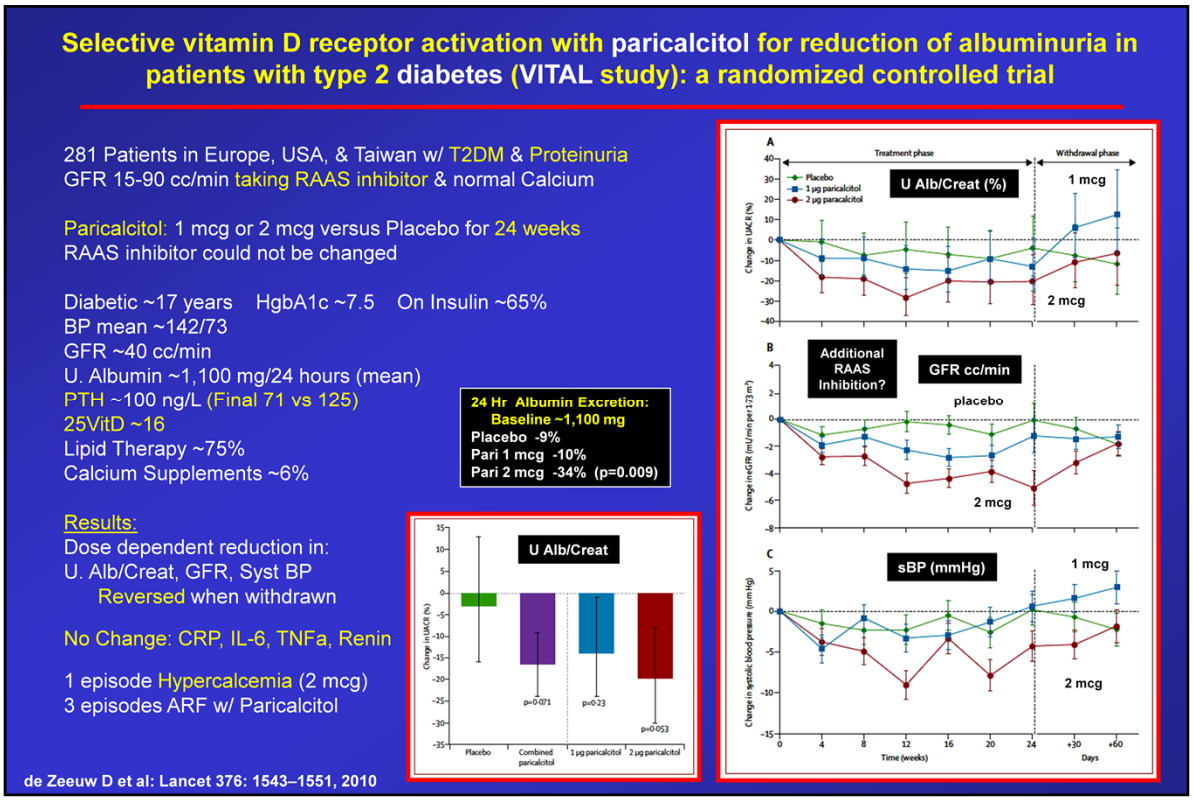
Table V: At the end of the study 134 pairs of biopsy specimens were available for analysis (124 after two years and 10 on withdrawal from the study after 5-17 months of treatment to start dialysis). These paired specimens came from 72 of the patients given alfacalcidol and 62 of the patients given placebo. The proportions of these patients with bone abnormalities at the start of the study were similar: 55 **(76%)** of those taking alfacalcidol and 45 **(73%)** of those taking placebo. At the end of the study, however, these proportions had changed to **54%** (39) of those taking alfacalcidol and **82%** (51) of those taking placebo.

In the minority of patients with apparently normal bone histology at the start of the study there was no significant difference in bone histology at the end of the study between those given alfacalcidol and those given placebo (P=0-73). In contrast, among patients with histological abnormalities at the start of the study, 23 (**42%**) of the patients given **alfacalcidol** showed **normal** histological appearances at the end of the study compared with only two (**4%**) of those given **placebo** (P < 0.001). Table V shows that the patients with preexisting

histological abnormalities who were treated with alfacalcidol showed improvements in hyperparathyroid bone disease in terms of a decrease in the severity of marrow fibrosis and a decrease in bone turnover as indicated by a significant decrease in histological indices of bone resorption (including the eroded surface and active eroded surface) and a decrease in indices of bone formation (including the number of osteoblasts, osteoblast surface, and osteoid surface and volume). Osteomalacia,

though uncommon, also improved, as indicated by a decrease in the maximum number of osteoid lamellae and in the osteoid thickness. In the group given placebo these histological indices tended to worsen (table V). No significant differences were seen between centers in the histological responses recorded.

At the end of the study adynamic bone lesions had resolved in four of the six patients taking alfacalcidol who had been affected at the start of the study and in two of the three patients taking placebo. Adynamic bone lesions developed in eight patients given alfacalcidol and in four patients given placebo. None of the patients with adynamic bone lesions at the start or end of the study had positive staining for aluminum at the mineral-osteoid interface. At the end of the study aluminum staining was no longer present in the two patients taking alfacalcidol in whom it had been present at the start of the study, but it appeared in one other patient taking alfacalcidol and in two patients taking placebo.



Abstract

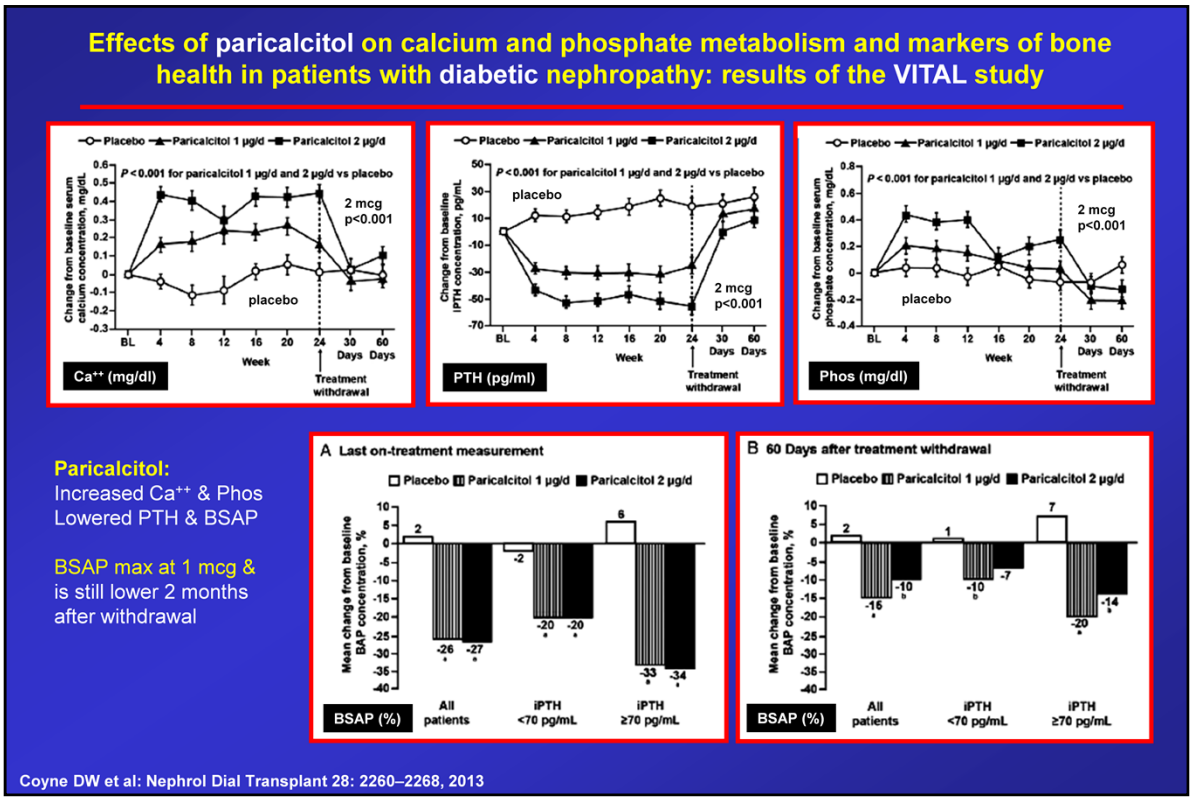
BACKGROUND: Despite treatment with renin–angiotensin–aldosterone system (RAAS) inhibitors, patients with diabetes have increased risk of progressive renal failure that correlates with albuminuria. We aimed to assess whether paricalcitol could be used to reduce albuminuria in patients with diabetic nephropathy.

METHODS: In this multinational, placebo-controlled, double-blind trial, we enrolled patients with type 2 diabetes and albuminuria who were receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Patients were assigned (1:1:1) by computer-generated randomization sequence to receive 24 weeks' treatment with placebo,1 µg/day paricalcitol, or 2 µg/day paricalcitol. The primary endpoint was the percentage change in geometric mean urinary albumin-to-creatinine ratio (UACR) from baseline to last measurement during treatment for the combined paricalcitol groups versus the placebo group. Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00421733.

FINDINGS: Between February, 2007, and October, 2008, 281 patients were enrolled and assigned to receive placebo (n=93), 1 µg paricalcitol (n=93), or 2 µg paricalcitol (n=95); 88 patients on placebo, 92 on 1 µg paricalcitol, and 92 on 2 µg paricalcitol received at least one dose of study drug, and had UACR data at baseline and at least one time point during treatment, and so were included in the primary

analysis. Change in UACR was: –3% (from 61 to 60 mg/mmol; 95% CI –16 to 13) in the placebo group; –16% (from 62 to 51 mg/mmol; –24 to –9) in the **combined paricalcitol groups**, with a between-group difference versus placebo of **–15%** (95% CI –28 to 1; **p=0.071**); –14% (from 63 to 54 mg/mmol; –24 to –1) in the 1 µg paricalcitol group, with a between-group difference versus placebo of –11% (95% CI –27 to 8; p=0.23); and **–20%** (from 61 to 49 mg/mmol; –30 to –8) in the 2 µg paricalcitol group, with a between-group difference versus placebo of –18% (95% CI –32 to 0; p=0.053). Patients on **2 µg paricalcitol** showed a nearly, sustained reduction in UACR, ranging from –18% to –28% (**p=0.014** vs placebo). Incidence of hypercalcaemia, adverse events, and serious adverse events was similar between groups receiving paricalcitol versus placebo.

INTERPRETATION: Addition of 2 µg/day paricalcitol to RAAS inhibition safely lowers residual albuminuria in patients with diabetic nephropathy, and could be a novel approach to lower residual renal risk in diabetes.



Coyne DW et al: Nephrol Dial Transplant 28: 2260–2268, 2013

Abstract

BACKGROUND: Chronic kidney disease (CKD) is associated with elevations in serum phosphate, calcium-phosphorus product and bone-specific alkaline phosphatase (BAP), with attendant risks of cardiovascular and bone disorders. Active vitamin D can suppress parathyroid hormone (PTH), but may raise serum calcium and phosphate concentrations. Paricalcitol, a selective vitamin D activator, suppressed PTH in CKD patients (stages 3 and 4) with secondary hyperparathyroidism (SHPT) with minimal changes in calcium and phosphate metabolism.

METHODS: The VITAL study enrolled patients with CKD stages 2-4. We examined the effect and relationship of paricalcitol to calcium and phosphate metabolism and bone markers in a post hoc analysis of VITAL. The study comprised patients with diabetic nephropathy enrolled in a double-blind, placebo-controlled, randomized trial of paricalcitol (1 or 2 µg/day). Urinary and serum calcium and phosphate, serum BAP, and intact PTH (iPTH) concentrations were measured throughout the study.

RESULTS: Baseline demographics and calcium, phosphate, PTH (49% with iPTH <70 pg/mL), and BAP concentrations were similar between groups. A transient, modest yet significant increase in phosphate was observed for paricalcitol 2 µg/day

(+0.29 mg/dL; $P < 0.001$). Dose-dependent increases in serum and urinary calcium were observed; however, there were few cases of hypercalcemia: one in the 1-µg/day group (1.1%) and three in the 2-µg/day group (3.2%). Significant reductions in BAP were observed that persisted for 60 days after paricalcitol discontinuation ($P < 0.001$ for combined paricalcitol groups versus placebo). Paricalcitol dose-dependent reductions in iPTH were observed. Paricalcitol in CKD patients (±SHPT) was associated with modest increases in calcium and phosphate.

CONCLUSION: Paricalcitol reduces BAP levels, which **may be beneficial for reducing vascular calcification**.

Effects Vitamin D Therapy and Cardiac Structure and Function in Patients With Chronic Kidney Disease: The PRIMO Randomized Controlled Trial

Paricalcitol (2 mcg) for 48 mths: 227 CKD patients w/ LVH, GFR 15-60 ml/min → LV mass index **(MRI)**
Half DM, 90% on RAAS inhibitors, BP ~ 135/75, normal Ca^{++} & Phos, Creat ~2.0, UACR 223 v 119, EF ~64%

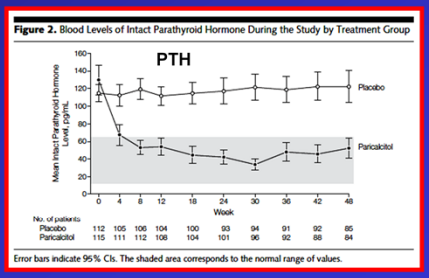


Table 5. Cardiovascular Hospitalizations by Treatment Group

Reason for Hospitalization	No. of Participants/ Cardiovascular Events	Follow-up, No. of Person-Years	Event Rate per 100 Person-Years	Study Day at Hospitalization
Placebo group	7/8	91.0	8.8	
Congestive heart failure[a]				16
Congestive heart failure				104
Chest pain				135
Chest pain				163
Aortic dissection				179
Congestive heart failure[a]				241
Congestive heart failure				289
Congestive heart failure				361
Paricalcitol group	1/1	94.3	1.1	
Chest pain				22
P value	.03		.04	

[a] These 2 hospitalizations were for the same participant.

Table 3. Repeated-Measures Analysis of Change in Cardiovascular Magnetic Resonance Imaging Measures From Baseline to 24 and 48 Weeks (Intention-to-Treat Population)[a]

Measures	24 Weeks			48 Weeks			Overall
	Paricalcitol (n = 104)	Placebo (n = 98)	*P* Value[b]	Paricalcitol (n = 88)	Placebo (n = 91)	*P* Value[b]	*P* Value[c]
Left ventricular mass index, g/m$^{2.7}$	0.27 (−0.15 to 0.68)	−0.15 (−0.57 to 0.27)	.05	0.34 (−0.14 to 0.83)	−0.07 (−0.55 to 0.42)	.15	.06
Left ventricular end-systolic volume index, mL/m$^{2.7}$	0.04 (−0.67 to 0.76)	0.002 (−0.72 to 0.72)	.91	0.58 (−0.25 to 1.41)	0.57 (−0.24 to 1.39)	.98	.94
Left ventricular end-diastolic volume index, mL/m$^{2.7}$	0.18 (−0.66 to 1.01)	−0.31 (−1.16 to 0.53)	.26	0.30 (−0.64 to 1.25)	−0.36 (−1.30 to 0.58)	.21	.19
Left ventricular ejection fraction, %	0.64 (−0.70 to 1.98)	0.28 (−1.07 to 1.64)	.61	0.62 (−0.90 to 2.14)	−0.54 (−2.06 to 0.98)	.18	.27

Paricalcitol v Placebo:
BNP +21% v +41% (ns)
HyperCa 23% v 1%

Thadhani, R et al: JAMA 307(7):674-684, 2012

Abstract

CONTEXT: Vitamin D is associated with decreased cardiovascular-related morbidity and mortality, possibly by modifying cardiac structure and function, yet firm evidence for either remains lacking.

OBJECTIVE: To determine the effects of an active vitamin D compound, paricalcitol, on left ventricular mass over 48 weeks in patients with an estimated glomerular filtration rate of 15 to 60 mL/min/1.73 m(2).

DESIGN, SETTING, AND PARTICIPANTS: Multinational, double-blind, randomized placebo-controlled trial among 227 patients with chronic kidney disease, mild to moderate left ventricular hypertrophy, and preserved left ventricular ejection fraction, conducted in 11 countries from July 2008 through September 2010.

INTERVENTION: Participants were randomly assigned to receive oral paricalcitol, 2 µg/d (n =115), or matching placebo (n = 112).

MAIN OUTCOME MEASURES: Change in left ventricular mass index over 48 weeks by cardiovascular magnetic resonance imaging. Secondary end points

included echocardiographic changes in left ventricular diastolic function.

RESULTS: Treatment with paricalcitol reduced parathyroid hormone levels within 4 weeks and maintained levels within the normal range throughout the study duration. At 48 weeks, the change in left ventricular mass index did not differ between treatment groups (paricalcitol group, 0.34 g/m(2.7) [95% CI, -0.14 to 0.83 g/m(2.7)] vs placebo group, -0.07 g/m(2.7) [95% CI, -0.55 to 0.42 g/m(2.7)]). Doppler measures of diastolic function including peak early diastolic lateral mitral annular tissue velocity (paricalcitol group, -0.01 cm/s [95% CI, -0.63 to 0.60 cm/s] vs placebo group, -0.30 cm/s [95% CI, -0.93 to 0.34 cm/s]) also did not differ. Episodes of hypercalcemia were more frequent in the paricalcitol group compared with the placebo group.

CONCLUSION: Forty-eight week therapy with paricalcitol **did not alter left ventricular mass index or improve certain measures of diastolic dysfunction** in patients with chronic kidney disease.

Effects Effect of Paricalcitol on Left Ventricular Mass and Function in CKD: The OPERA Trial

Wang AYM et al: J Am Soc Nephrol 25:175–186, 2014

Paricalcitol (1 mcg) for 52 wks:

60 CKD (stage 3-5) patients w/ LVH
→ LV mass index **(MRI)**
30% DM, 82% on RAAS inhibitors
BP ~ 133/75, normal Ca^{++} & Phos
GFR ~22 cc/min, EF ~68%

Paricalcitol v Placebo:

LV Mass Index:	-2.59	-4.85	p=0.40
LV EF:	+0.45	-0.80	p=0.80
Deceleration Time:	+23.0	-3.5	p=0.05

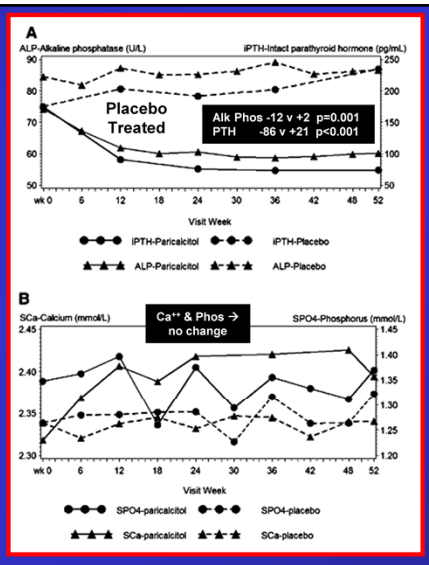


Table 5. A summary of adverse events in study subjects

Adverse Events	Paricalcitol (*n*=30)	Placebo (*n*=30)	*P*
Patients with hospitalizations, *n* (%)	2 (0.07)	10 (0.33)	0.02
Total hospitalization episodes	5	14	
Patients with cardiovascular-related hospitalizations	0 (0)	5 (16.67)	
Hospitalization days, median (range)	0 (0 to 14)	0 (0 to 55)	0.02
Patients with hypercalcemia, *n* (%)	13 (43.3)	1 (3.3)	<0.001
Cardiovascular events, number of episodes	0	6 (5 patients)	

Vitamin D seems to protect against cardiovascular disease, but the reported effects of vitamin D on patient outcomes in CKD are controversial. We conducted a prospective, double blind, randomized, placebo controlled trial to determine whether oral activated vitamin D reduces left ventricular (LV) mass in patients with stages 3–5 CKD with LV hypertrophy.

Subjects with echocardiographic criteria of LV hypertrophy were randomly assigned to receive either oral paricalcitol (1 mg) one time daily (n=30) or matching placebo (n=30) for 52 weeks. The primary end point was change in LV mass index over 52 weeks, which was measured by cardiac magnetic resonance imaging. Secondary end points included changes in LV volume, echocardiographic measures of systolic and diastolic function, biochemical parameters of mineral bone disease, and measures of renal function. Change in **LV mass index did not differ significantly between groups** (median [interquartile range], 22.59 [26.13 to 0.32] g/m2 with paricalcitol versus 24.85 [29.89 to 1.10] g/m2 with placebo). Changes in LV volume, ejection fraction, tissue Doppler-derived measures of early diastolic and systolic mitral annular velocities, and ratio of early mitral inflow velocity to early diastolic mitral annular velocity did not differ between the groups. However, paricalcitol treatment **significantly reduced intact parathyroid hormone** (P=0.001) and **alkaline phosphatase** (P=0.001) levels as well as the number of **cardiovascular-related hospitalizations** compared with placebo.

In conclusion, 52 weeks of treatment with oral paricalcitol (1 mg one time daily) significantly improved secondary hyperparathyroidism but did not alter measures of LV structure and function in patients with severe CKD.

Effects Paricalcitol and Endothelial Function in Chronic Kidney Disease Trial (PENNY)

Paricalcitol (2 mcg) for 12 wks: 88 CKD patients w/ PTH >65 pg/ml, Stage 3 & 4 → Flow-Medicated Dilitation **(FMD)**
Glucose ~108, DM 31%, BMI ~29, BP ~ 125/73, Ca ~8.9, Phos ~3.8, PTH ~102, 25VitD ~14 ng/ml, GFR ~32

Table 2. Within- and Between-Group Changes (12th Week to Baseline) in Biomarkers of Mineral Metabolism, in Glucose, in Lipids, and in Plasma Renin Activity, Aldosterone, and C-Reactive Protein

Variables	Paricalcitol	Placebo	Difference (Paricalcitol vs Placebo)	*P* Value
Bone mineral metabolism biomarkers				
Parathormone, pg/mL	−75 (−90 to −60)	21 (5–36)	−96 (−117 to −74)	<0.001
Calcium, mmol/L +0.3 mg/dl	0.069 (0.031–0.11)	0.002 (−0.033 to 0.036)	0.067 (0.016–0.118)	0.01
Phosphate, mmol/L +0.4 mg/dl	0.13 (0.07–0.19)	0.05 (0.0018–0.10)	0.08 (0.004–0.16)	0.039
FGF23, pg/mL	107 (44–170)	−20 (−64 to 24)	127 (51–203)	0.001
1,25-OH_2 vitamin D, pmol/L	−62.4 (−78.0 to −49.4)	−13.0 (−26.0 to −2.6)	49.0 (30.7–67.3)	<0.001
25-OH vitamin D, nmol/L	5.1 (0.8–9.4)	2.0 (−4.0 to 8.1)	3.0 (−10.4 to 4.3)	0.41
Glucose, mmol/L	0.13 (−0.32 to 0.57)	0.27 (−0.39 to 0.94)	−0.14 (−0.98 to 0.64)	0.72
LDL cholesterol, mmol/L	0.023 (−0.207 to 0.254)	−0.127 (−0.433 to 0.176)	0.15 (−0.24 to 0.50)	0.48
HDL cholesterol, mmol/L	0.023 (−0.047 to 0.096)	−0.023 (−0.088 to 0.041)	0.046 (−0.05 to 0.14)	0.33
Triglycerides, mmol/L	0.058 (−0.137 to 0.251)	0.071 (−0.053 to 0.196)	−0.013 (−0.55 to 0.24)	0.91
PRA, μg/L per hour	2.9 (−72.1 to 77.8)	21.6 (−67.0 to 110.0)	−18.7 (−131.3 to 94.8)	0.75
Aldosterone, nmol/L	−0.36 (−1.13 to 0.42)	0.03 (−0.66 to 0.69)	−0.39 (−1.37 to 0.65)	0.48
CRP, mg/L	1.3 (−0.6 to 3.2)	0.4 (−4.4 to 5.2)	0.9 (−4.0 to 6.0)	0.71

Values are mean and 95% confidence intervals. CRP indicates C-reactive protein; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PRA, plasma renin activity.

Zoccali C et al: Hypertension 64:1005-1011, 2014

Abstract: Altered vitamin D metabolism and low levels of the active form of this vitamin, 1,25-dihydroxy-vitamin D, is a hallmark of chronic kidney disease (CKD), but there is still no randomized controlled trial testing the effect of active forms of vitamin D on vascular function in patients with CKD. Paricalcitol and endothelial function in chronic kidney disease (PENNY) is a double-blinded randomized controlled trial (ClinicalTrials.gov, NCT01680198) testing the effect of an active form of vitamin D, paricalcitol (2 μg/d×12 weeks) on endothelium-dependent and endothelium-independent vasodilatation in 88 patients with stage 3 to 4 CKD and parathormone >65 pg/mL (paricalcitol, n=44; placebo, n=44).

Paricalcitol treatment reduced **parathormone (−75 pg/mL**; 95% confidence interval, –90 to –60), whereas parathormone showed a small rise during placebo (21 pg/mL; 95% confidence interval, 5–36). Blood pressure did not change in both study arms. Baseline flow-mediated dilation was identical in patients on paricalcitol (3.6±2.9%) and placebo (3.6±2.9%) groups. After 12 weeks of treatment, **flow-mediated dilation rose in the paricalcitol** but not in the placebo group, and the between group difference in flow-mediated dilation changes (the primary end point, **1.8%**; 95% confidence interval, 0.3–3.1%) was significant (***P*=0.016**), and the mean proportional change in flow-mediated dilation was **61% higher** in paricalcitol treated patients than in placebo-treated patients. Such an effect was abolished 2 weeks after stopping the treatment. No effect of paricalcitol on endothelium-independent vasodilatation was registered.

Paricalcitol improves endothelium dependent vasodilatation in patients with stage 3 to 4 CKD. Findings in this study support the hypothesis that vitamin D may exert favorable effects on the cardiovascular system in patients with CKD.

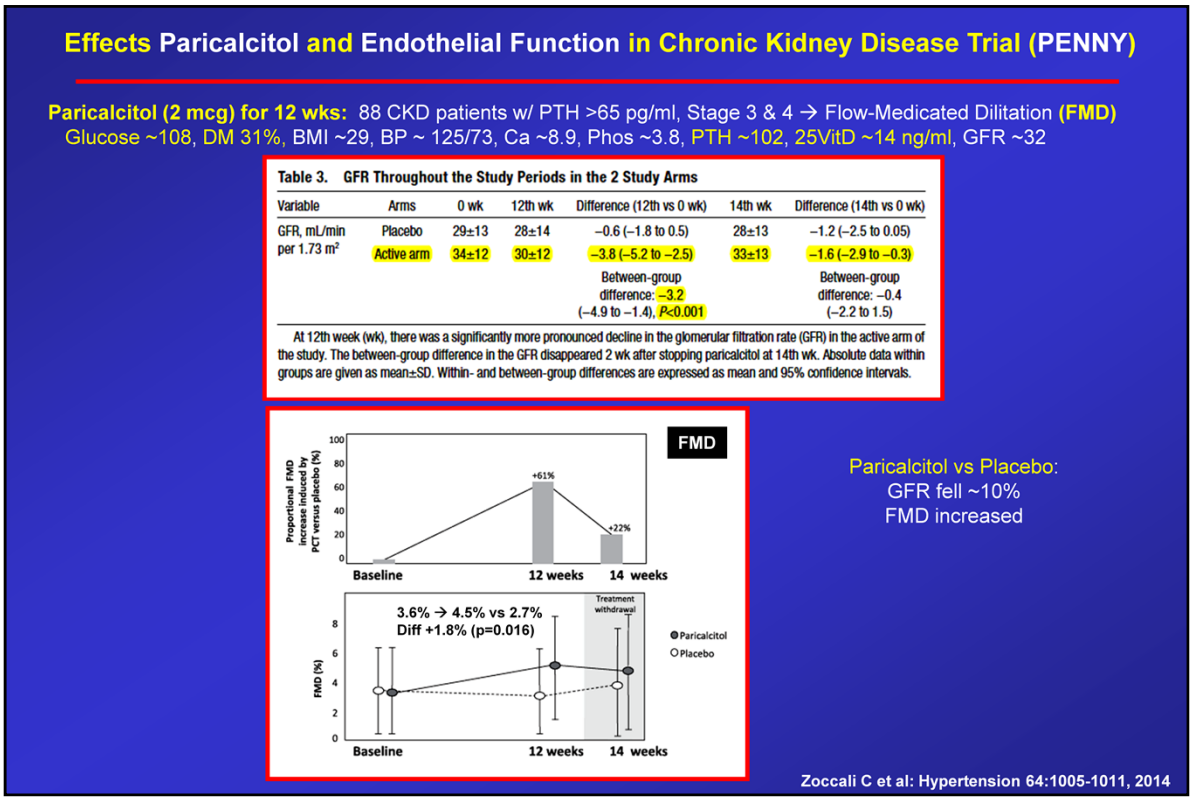
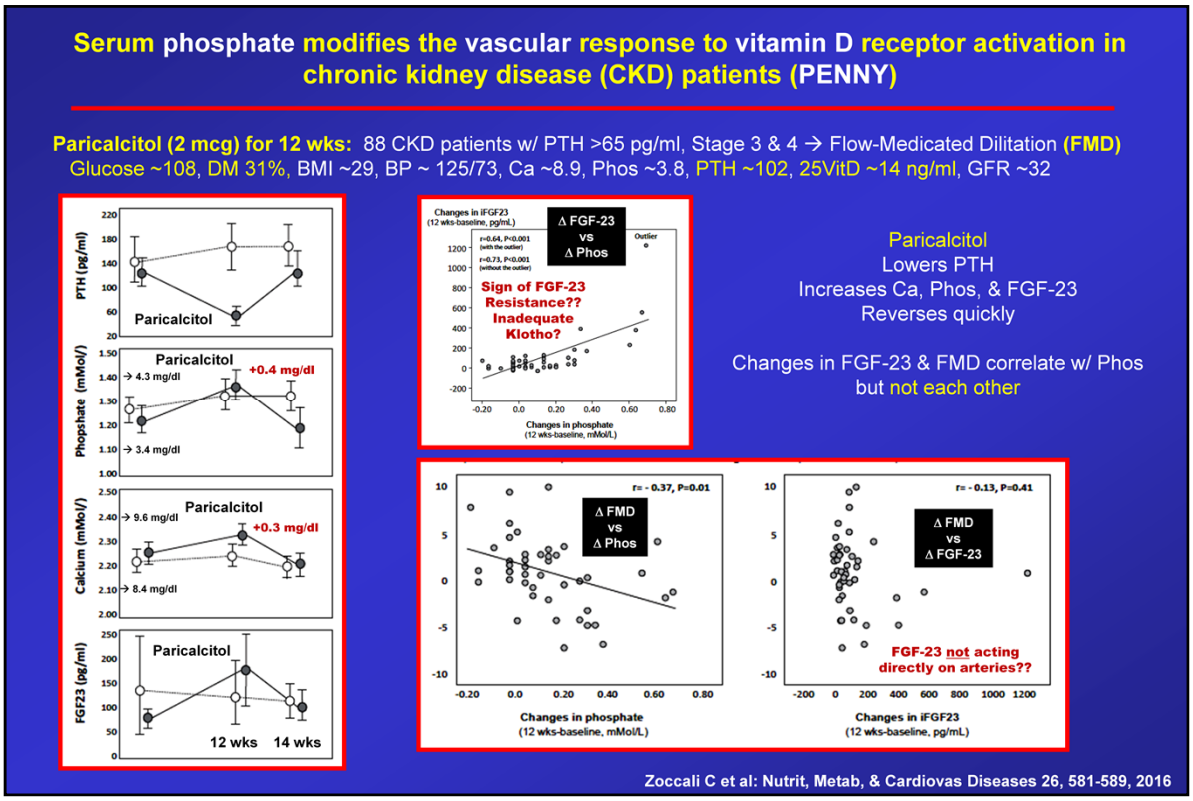


Figure 2. Endothelium-dependent (flow-mediated dilation [FMD]) vasodilation at baseline, after 12 weeks of treatment, and 2 weeks after stopping treatment (14th weeks) in patients receiving paricalcitol and placebo. Data are mean±SD. At 12th and 14th weeks, we also reported (**top**) the mean proportional change in FMD induced by paricalcitol (PCT) vs placebo group expressed as percentage.



Hughes comment: *1-25VitD stimulates both FGF-23 & Klotho but FGF-23 inhibits the production of Klotho so that the net effect is FGF-23 resistance and increasing phosphate!*

Abstract

BACKGROUND AND AIMS: Vitamin D receptor activation (VDRA) ameliorates endothelial dysfunction in CKD patients but also increases phosphate and FGF-23, which may attenuate the beneficial effect of VDRA on endothelial function.

METHODS AND RESULTS: This is a pre-specified secondary analysis of the PENNY trial (NCT01680198) testing the effect of phosphate and FGF-23 on the flow mediated vasodilatory (FMD) response to paricalcitol (PCT, 2 µg/day) and placebo over a 12-weeks treatment period. Eighty-eight stage 3-4 CKD patients were randomized to PCT (n = 44) and Placebo (n = 44). Endothelial function was assessed by measuring endothelium dependent forearm blood flow (FBF) response to ischemia. The FMD response was 61% higher in PCT treated patients than in those on placebo (P = 0.01). Phosphate (+11%, P = 0.039), calcium (+3%, P = 0.01) and, particularly, FGF23 (+164%, P < 0.001) increased in PCT treated patients. Changes in FMD induced by PCT were inversely associated with phosphate (r = -0.37, P = 0.01) but were independent of changes in FGF-23, calcium, and PTH. The response to PCT was maximal in patients with no changes in phosphate (1st tertile),

attenuated in those with mild-to-moderate rise in phosphate (2nd tertile), and abolished in those with the most pronounced phosphate increase (3rd tertile) (effect modification P = 0.009). No effect modification by FGF-23 and other variables was observed.

CONCLUSIONS: The beneficial effect of **PCT on endothelial function in CKD is maximal in patients with no or minimal changes in phosphate** and it is abolished in patients with a pronounced phosphate rise. These findings generate the hypothesis that the endothelium protective effect by VDRA may be potentiated by phosphate lowering interventions.

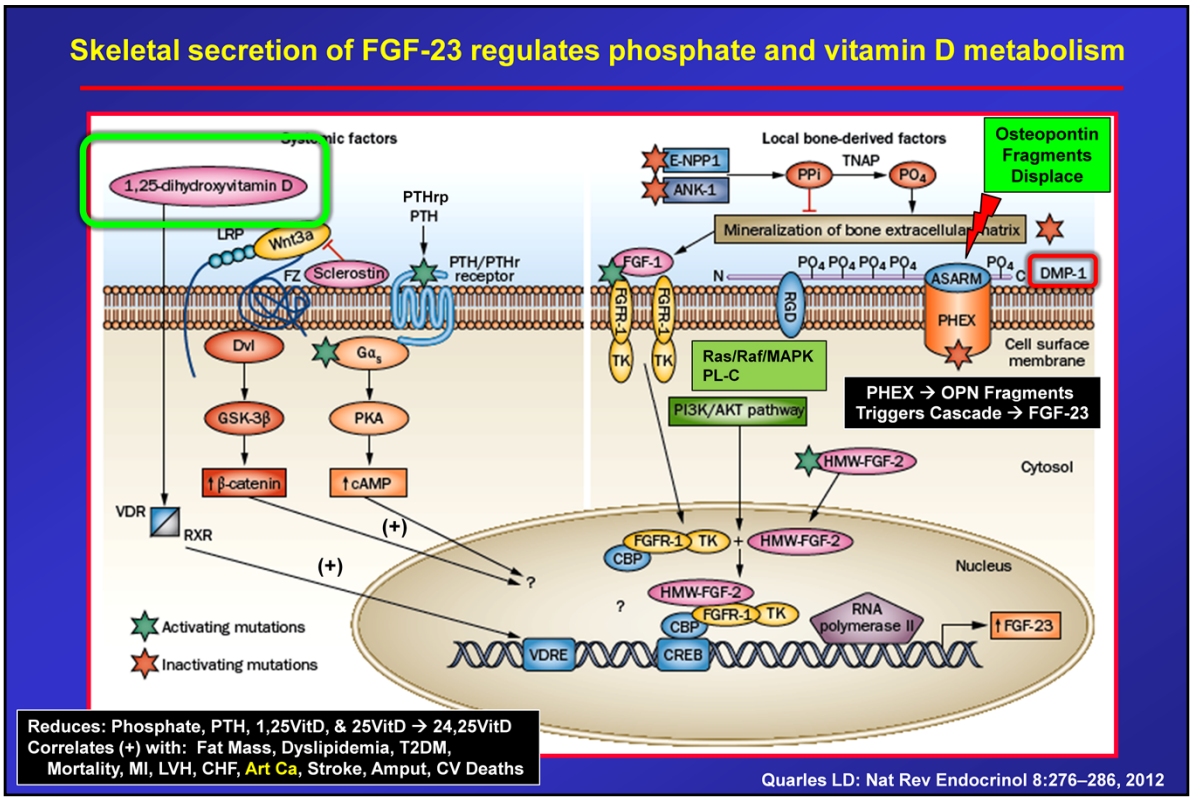
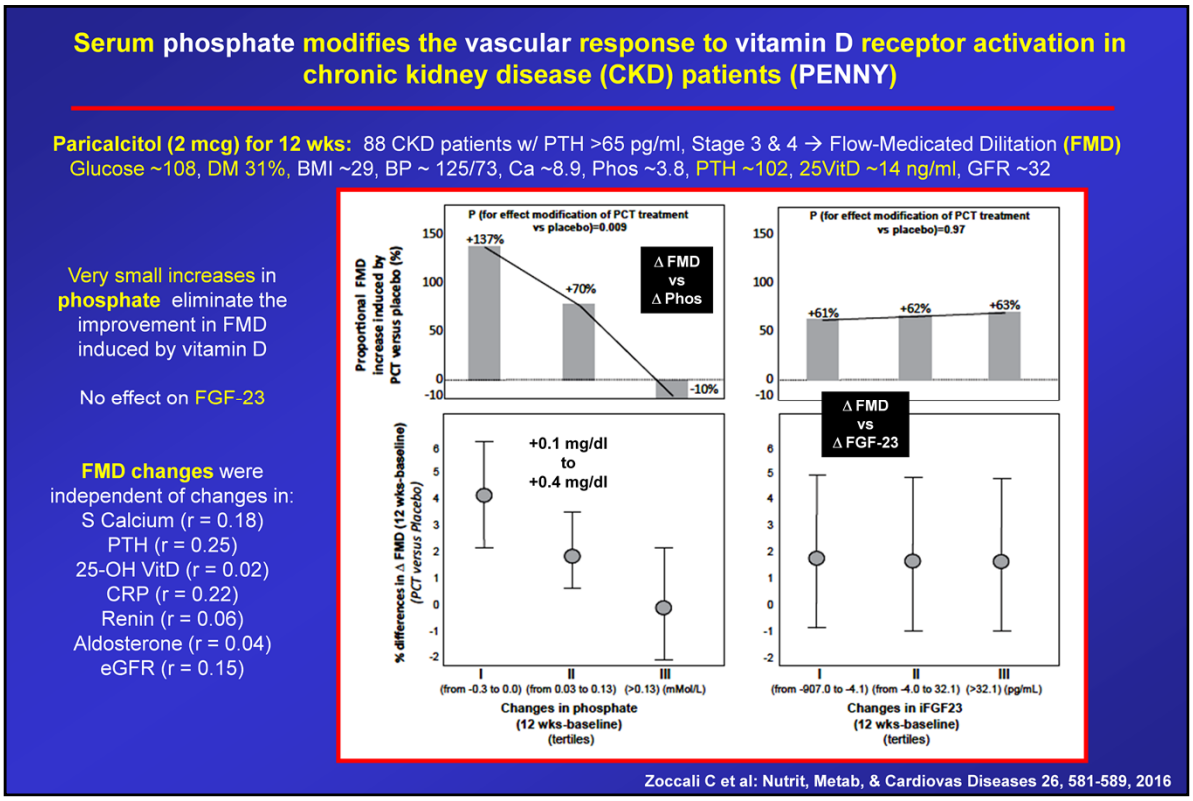


Figure 1 | A speculative model of *FGF23* gene transcriptional regulation. Four activating mutations or pathways (involving FGFR-1, Gαs encoded by *GNAS*, PTH/PTHr receptor and HMW-FGF-2) and four inactivating mutations (involving PHEX, DMP-1, E-NPP1 and ANK-1) are associated with increased FGF-23 expression in bone. Local bone-derived factors that are linked to mineralization are shown on the right-hand side. ANK-1 and E-NPP1 regulate the transport and biosynthesis of pyrophosphate, and TNAP regulates the conversion of pyrophosphate to phosphate in the extracellular matrix mineralization process, whereas both **PHEX** and **DMP-1** regulate bone mineralization through mechanisms that remain to be fully elucidated. Evidence exists in osteoblasts derived from the Hyp mouse model that defective mineralization is linked to the activation of FGFR-1 as well as **HMW-FGF-2** integrative nuclear signaling pathways. The left-hand side of the figure shows systemic factors involved in FGF-23 regulation. 1,25-dihydroxyvitamin D is an important regulator of FGF-23 expression, acting through the VDR and VDRE. PTH can also stimulate FGF-23 through a sclerostin-dependent mechanism involving the Wnt–β-catenin pathway, or through stimulation of GNAS and cAMP-dependent signaling pathways, as well as indirectly through stimulation of 1,25-dihydroxyvitamin D. Intrinsic and systemic factors are integrated at the levels of *cis*-acting elements in the proximal *FGF23* promoter that remain to be elucidated. A question mark (?) indicates areas of uncertainty. Abbreviations: Hyp, mouse model of X-linked hypophosphatemic rickets; PO4, phosphate; PPi, pyrophosphate; TK, tyrosine kinase.

Abstract The discovery of fibroblast growth factor 23 (FGF-23) has expanded our understanding of phosphate and vitamin D homeostasis and provided new insights into the pathogenesis of hereditary hypophosphatemic and hyperphosphatemic disorders, as well as acquired disorders of phosphate metabolism, such as chronic kidney disease. FGF-23 is secreted by **osteoblasts and osteocytes** in bone and principally **targets the kidney** to regulate the reabsorption of phosphate, the production and catabolism of 1,25-dihydroxyvitamin D and the expression of **α-Klotho**, an anti-ageing hormone. Secreted FGF-23 plays a central role in complex endocrine networks involving local bone-derived factors that regulate mineralization of extracellular matrix and systemic hormones involved in mineral metabolism. Inactivating mutations of PHEX, DMP1 and ENPP1, which cause hereditary hypophosphatemic disorders and primary defects in bone mineralization, stimulate FGF23 gene transcription in osteoblasts and osteocytes, at least in part, through canonical and intracrine FGF receptor pathways. These FGF-23 regulatory pathways may enable systemic phosphate and vitamin D homeostasis to be coordinated with bone mineralization. FGF-23 also functions as a counter-regulatory hormone for 1,25-dihydroxyvitamin D in a bone-kidney endocrine loop. FGF-23, through regulation of additional genes in the kidney and extra-renal tissues, probably has broader physiological functions beyond regulation of mineral metabolism that account for the association between FGF-23 and increased mortality and morbidity in chronic kidney disease.



Formal effect modification analysis showed that among patients within the 1st tertile of serum phosphate changes, the absolute FMD increase was on average 4% higher in PCT treated than in placebo treated patients corresponding to a 137% proportionally higher increase in FMD (Fig. 5, left panels). The difference in FMD increase between PCT and placebo was still relevant (2% in absolute terms and 70% in proportional terms) in the 2nd tertile but null in those with serum phosphate changes in the 3rd tertile (P for the effect modification = 0.009, see Fig. 5, left panels). Data adjustment for baseline eGFR and phosphate binders use did not affect the strength of this interaction which remained highly significant (P = 0.01). Changes in FGF-23 (12 weeks baseline) did not modify the simultaneous changes in FMD induced by PCT versus placebo (Fig. 5), the difference in absolute and proportional χηανγε ιν FMD between PCT treated and placebo treated patients being almost identical throughout tertiles of change in FGF-23 (Fig. 5, right panel). FMD changes were independent of changes in serum calcium (r = 0.18, P = 0.25), PTH (r = 0.25, P = 0.10), 25-OHVitD (r = 0.02, P = 0.91), CRP (rho = 0.22, P = 0.15), Renin (r = 0.06, P = 0.70), aldosterone (r = 0.04, P = 0.81) and eGFR (r = 0.15, P = 0.32) and there was no effect modification by these parameters on FMD changes during the trial (P ranging from 0.10 to 0.80).

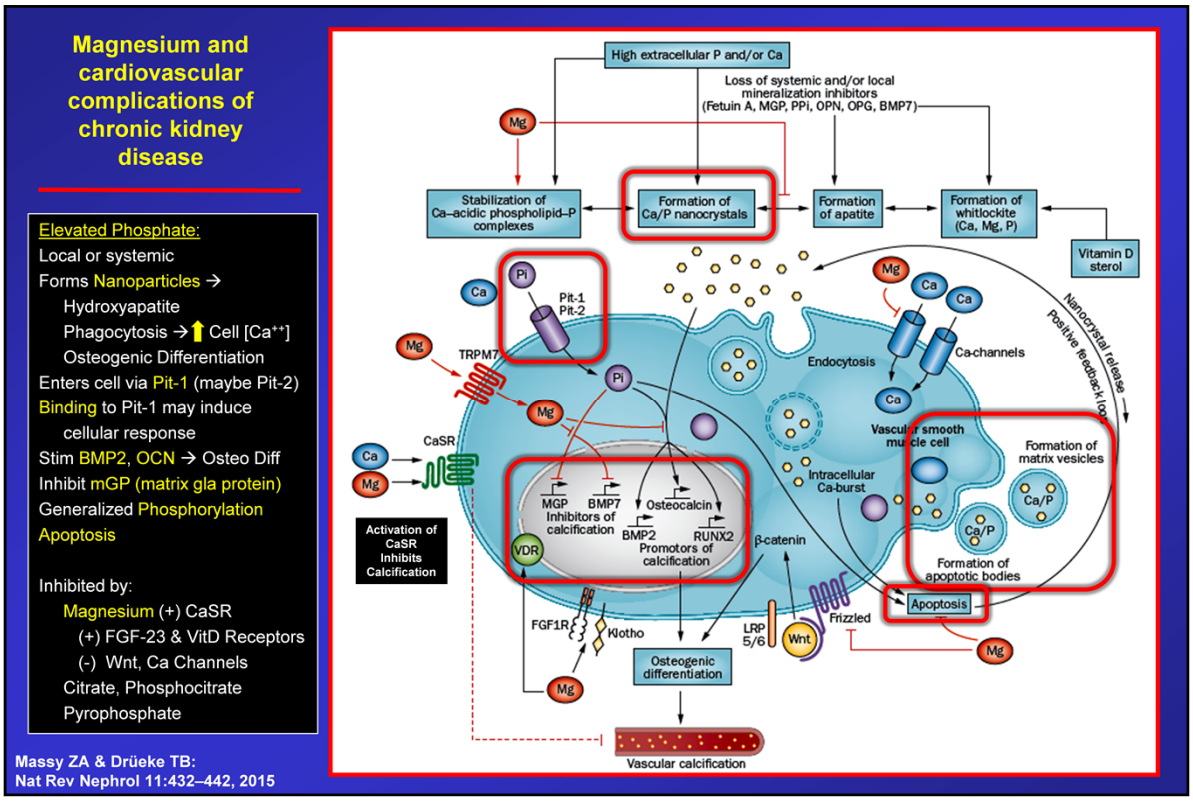


Figure 1 | The putative inhibitory effects of magnesium on the process of vascular calcification. Abnormalities in mineral metabolism, particularly hyperphosphataemia, and loss of inhibitors of mineralization leads to the formation and deposition of Ca/P nanocrystals, which are taken up by VSMCs. Lysosomal degradation of the endocytosed crystals results in intracellular release of Ca and Pi. In addition, Pi accumulates in the cell via uptake through Pit-1 and probably also Pit-2. To compensate for excess Ca/P, VSMCs form matrix vesicles loaded with Ca/P products and the mineralization inhibitors. The intracellular Ca-burst induced by endocytosed nanocrystals and Pi uptake triggers apoptosis, resulting in the formation of Ca/P-containing apoptotic bodies. Matrix vesicles and apoptotic bodies cause a positive feedback loop through nanocrystal release into the surrounding milieu, thus amplifying the calcification process. Furthermore, Ca/P nanocrystals and Pi induce the expression of genes that promote the calcification–mineralization process and repress the expression of factors that inhibit calcification, resulting in transdifferentiation of VSMCs to osteoblast-like cells and, ultimately, vessel calcification. Mg interferes with the process of vascular calcification by inhibiting transformation of amorphous Ca/P to apatite and by forming Mg-substituted whitlockite crystals, which result in smaller, more soluble deposits. Secondly, Mg functions as a Ca-channel antagonist and thus inhibits the entry of Ca into the cells. Thirdly, Mg enters the cell via TRPM7 and restores the balance between expression of calcification promoters and inhibitors by neutralizing phosphate-induced inhibition of MGP and BMP7 and enhanced expression of RUNX2 and BMP2. These effects prevent osteoblastic conversion and calcification

of VSMCs. In addition, Mg acts on the CaSR; activation of this receptor by calcimimetics has been shown to inhibit VSMC calcification but the molecular mechanisms have not yet been identified.

Abbreviations: BMP, bone morphogenetic protein; Ca, calcium; CaSR, calcium-sensing receptor; FGF1R, fibroblast growth factor receptor-1; LRP 5/6, LDL receptor-related protein 5/6; Mg, magnesium; MGP, matrix gla protein; OPG, osteoprotegerin; OPN, osteopontin; Pi, inorganic phosphate; Pit, sodium-dependent phosphate transporter; PPi, pyrophosphate; RUNX2, runt-related transcription factor 2; TRPM7, transient receptor potential cation channel subfamily M member 7; VDR, vitamin D receptor; VSMC, vascular smooth muscle cell. Permission obtained from Oxford University Press © Massy, Z. A. & Drüeke, T. B. *Clin. Kidney J*. 5 (Suppl. 1), i52–i61 (2013).

Abstract Cardiovascular complications are the leading cause of death in patients with chronic kidney disease (CKD). Abundant experimental evidence suggests a physiological role of magnesium in cardiovascular function, and clinical evidence suggests a role of the cation in cardiovascular disease in the general population. The role of magnesium in CKD-mineral and bone disorder, and in particular its impact on cardiovascular morbidity and mortality in patients with CKD, is however not well understood. Experimental studies have shown that magnesium inhibits vascular calcification, both by direct effects on the vessel wall and by indirect, systemic effects. Moreover, an increasing number of epidemiologic studies in patients with CKD have shown associations of serum magnesium levels with intermediate and hard outcomes, including vascular calcification, cardiovascular events and mortality. Intervention trials in these patients conducted to date have had small sample sizes and have been limited to the study of surrogate parameters, such as arterial stiffness, vascular calcification and atherosclerosis. Randomized controlled trials are clearly needed to determine the effects of magnesium supplementation on hard outcomes in patients with CKD.

Bone, Artery, & Renal Function in CKD

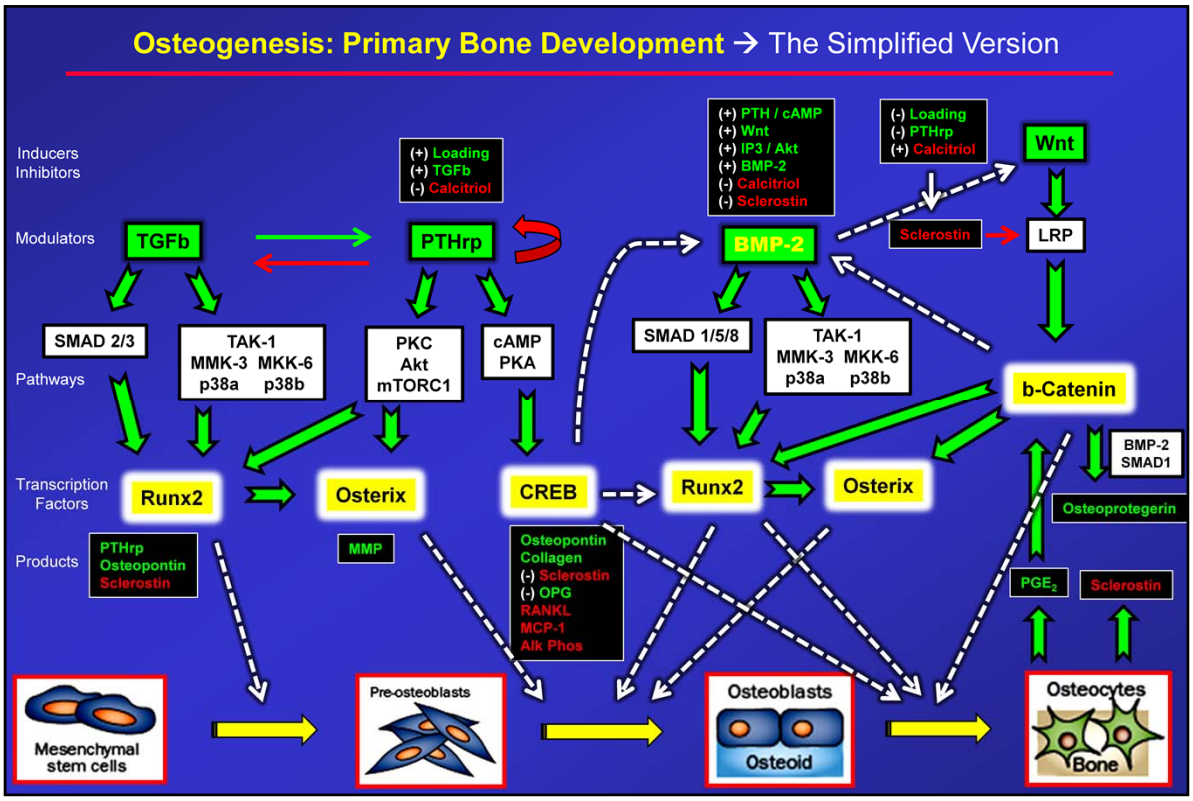
Supplementation of Vitamin D, Calcitriol, &/or Vitamin D Analogs

Observations:

1. Vitamin D supplementation:
    1. Lowers PTH, intracellular Ca^{++}, Adhesion Molecules, AGE-P, proteinuria
    2. Increases Serum Ca^{++}, 1-25 Vitamin D, FMD, microcirculatory vasodilatation, NOS
    3. Prevents increase in pulse pressure
    4. Tends to increase aKlotho & FGF-23 (data not shown)
    5. Does not change phosphorus or bone markers
2. Calcitriol supplementation
    1. Lowers Alk Phos, PTH, Renin, angiotensinogen, proteinuria
    2. Increases S & U Ca^{++}, bone density, & hepatic growth factor (**reduces renal fibrosis**)
    3. Combination w/ VitD: reduces mineralization rate, woven osteoid, blast & clast activity
    4. Does not change urinary phosphorus
3. Vitamin D analog supplementation
    1. Similar impact on Ca^{++}, Phos, PTH, & Alk Phos; similar incidence HyperCa & HyperPhos
    2. Reduces osteoid, blast, & clast activity
    3. Reduces GFR, proteinuria, BP, & hospitalization for CV events
    4. Increases FMD & FGF-23 → correlate w/ Phos; increase in Phos eliminates increase in FMD
    5. Increases Sclerostin by reducing PTH (data not shown)

Recommendations:

1. Patients with diabetes (everyone?) should have “normal” 25-Vitamin D levels (>40, maybe >60)
2. Low dose calcitriol (0.25 → 0.50 mcg daily – if S. Ca^{++} normal) should be initiated early in CKD (proteinuria)


Osteogenesis: Primary Bone Development → The Simplified Version
Inducers
Inhibitors
Modulators
Pathways
Transcription Factors
Products
(+) Loading
(+) TGFb
(-) Calcitriol
(+) PTH / cAMP
(+) Wnt
(+) IP3 / Akt
(+) BMP-2
(-) Calcitriol
(-) Sclerostin
(-) Loading
(-) PTHrp
(+) Calcitriol
Wnt
TGFb
PTHrp
BMP-2
Sclerostin
LRP
SMAD 2/3
TAK-1
MMK-3 MKK-6
p38a p38b
PKC
Akt
mTORC1
cAMP
PKA
SMAD 1/5/8
TAK-1
MMK-3 MKK-6
p38a p38b
b-Catenin
BMP-2
SMAD1
Osteoprotegerin
Runx2
Osterix
CREB
Runx2
Osterix
PTHrp
Osteopontin
Sclerostin
MMP
Osteopontin
Collagen
(-) Sclerostin
(-) OPG
RANKL
MCP-1
Alk Phos
PGE2
Sclerostin
Mesenchymal stem cells
Pre-osteoblasts
Osteoblasts
Osteoid
Osteocytes
Bone