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Impact of Activated Vitamin D on Insulin Resistance in Non-diabetic Chronic Kidney Disease Patients

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Abstract

Background—Although vitamin D deficiency has been associated with increased insulin resistance, a causal link has not been established. Interpreting the relationship has been confounded by a close correlation between vitamin D deficiency and obesity. The current clinical approach of assessing endogenous 25-hydroxyvitamin D (25(OH)D) concentrations in patients with Chronic Kidney Disease (CKD), and independently administering activated vitamin D (AD), allows a unique opportunity to clarify cause and effect in the relationship of vitamin D, obesity, and insulin resistance.

Methods—We assessed how 25(OH)D and body mass index (BMI) related to fasting insulin concentrations in 120 nondiabetic CKD patients. In addition, we described how treatment with AD modified these relationships.

Results—In the full cohort, fasting insulin concentrations varied inversely with both 25(OH)D ($r = -0.22$, $P = 0.02$) and BMI ($r = -0.36$, $P < .0001$). The administration of AD altered these relationships. In individuals treated with AD, there was no association between 25(OH)D and fasting insulin, and the mean fasting insulin concentrations were significantly lower than in those not receiving AD (40.5 ± 22.0 vs 54.1 ± 30.9 pmol/L, $P = 0.01$). In a multivariate analysis, both AD treatment and BMI were independent predictors of fasting insulin. Furthermore, obese patients treated with AD had insulin concentrations similar to nonobese patients (46.1 ± 24.9 vs 40.2 ± 21.5 pmol/L), whereas untreated obese patients had markedly higher fasting insulin concentrations (74.4 ± 33.4 pmol/L, $P = 0.003$).

Conclusion—25(OH)D deficiency is associated with insulin resistance in CKD. Replacement with pharmacologic doses of AD is associated with lower fasting insulin concentrations, especially in obese patients.

Index words

Vitamin D; 25(OH)D; insulin; obesity; BMI; Chronic Kidney Disease

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Introduction

There is growing evidence that vitamin D has an important role in energy metabolism and glucose homeostasis. However, although many cross sectional studies have demonstrated an association between vitamin D deficiency and insulin resistance(1–3), randomized trials have reached disparate conclusions(4–6), and causality has not been established. In addition, since vitamin D deficiency is strongly associated with obesity(7–9), the confounding effect of obesity further complicates the relationship between vitamin D and insulin resistance.

Derived from either skin metabolism or dietary sources, fat soluble cholecalciferol and ergocalciferol are metabolized by the liver into a more polar form, 25(OH)D. In a tightly regulated process, 25(OH)D is further hydroxylated into a biologically active form, 1,25-hydroxyvitamin D(1,25(OH)₂D), by renal 1-alpha hydroxylase. Although 1,25(OH)₂D is the active form, 25(OH)D concentrations are predominantly used to define vitamin D deficiency because of its longer half life and 1000 fold higher serum concentration. Due to a deficiency of renal 1-alpha hydroxylase activity, patients with CKD are considered at risk for 1,25(OH)₂D deficiency, as evidenced by compensatory hyperparathyroidism, despite adequate 25(OH)D availability. In the routine clinical care of CKD patients, both forms of vitamin D are frequently administered. Ergocalciferol is administered to treat low 25(OH)D concentrations, with a goal of greater than 75 nmol/L(10), and because 1,25(OH)₂D concentrations are not routinely assayed, AD is prescribed in response to secondary hyperparathyroidism.

The practice of administering the downstream active vitamin D metabolite provides an opportunity to better understand the relationship between 25(OH)D, obesity, and insulin resistance. We hypothesized that if vitamin D deficiency causes insulin resistance in CKD patients, then the administration of AD should improve insulin sensitivity, and should weaken any observed association between endogenous 25(OH)D concentrations and fasting insulin.

We evaluated the associations between 25(OH)D, BMI, and insulin resistance in a cohort of pre-dialysis CKD patients. We assessed whether treatment with AD was associated with a difference in fasting insulin concentrations and how treatment with AD modified the independent relationships between 25(OH)D and BMI to fasting insulin concentrations.

Methods

CKD patients seen in the ambulatory nephrology clinic at Beth Israel Deaconess Medical Center between Sept 1, 2009 and February 9, 2011 were evaluated for inclusion. Institutional Review Board approval was obtained for this study. Routine clinic schedules were reviewed, and potentially eligible patients were contacted the evening prior to the visit to discuss the study and to ask the patients to fast overnight. Clinic attendees were screened in a consecutive fashion. The approximate response rate was 40 percent; the primary reason for non participation was reluctance to fast overnight. In order to minimize the potential confounding influence of diabetes, we included only nondiabetic patients, as defined by a fasting glucose < 126 mg/dl or no history of diabetes per electronic chart review. Patients requiring dialysis were not eligible. 125 patients agreed to participate, and written consent was obtained at the time of the clinic visit. Five were excluded due to lack of fasting prior to serum collection. Assessments included the recording of demographic and anthropomorphic data, including seated blood pressure, height and weight. Morning plasma and serum were obtained after eight hours of overnight fasting. Routine laboratory data and the medication profiles were extracted from the patient's electronic database. Urine samples were not collected. Estimated Glomerular Filtration Rates (eGFR) were calculated using the

Modification of Diet in Renal Disease equation, which uses the serum creatinine concentration, age, race, and gender of the patient. Insulin concentrations were measured by ELISA (Millipore, Billerica, MA), with an intra-assay coefficient of variation (CV) of 4.6–7.0 percent. Fasting 25(OH)D concentrations (Immunodiagnostik AG, Bensheim, Germany) were measured by ELISA. Overall assay performance was 7% (both intra-assay and inter-assay CVs), with a 100% cross reactivity with 25(OH)D₃ and 67.8% cross reactivity with 25(OH)D₂.

Patients were prescribed AD at the discretion of their treating nephrologists. Current clinical guidelines dictate initiation of AD in response to secondary hyperparathyroidism, with CKD stage specific guidelines (CKD III, PTH 3.85–7.7 pmol/L; CKD IV, PTH 7.7–12.1 pmol/L) (11). AD dosing was adjusted to maintain PTH concentrations in range, while avoiding hypercalcemia or hyperphosphatemia, as part of routine care.

Pairwise correlation coefficients were calculated between fasting insulin and 25(OH)D concentrations, BMI, age, eGFR, systolic blood pressure, and parathyroid (PTH) concentrations. The cohort was stratified by administration of AD. Baseline characteristics were compared using chi-squared tests for categorical variables and analysis of variance or t-tests for continuous variables. Wilcoxon Rank Sums test was used for comparison of PTH, serum creatinine, and eGFR given significant deviation from a normal distribution for these variables. In order to assess how treatment with AD affected the relationship between 25(OH)D concentrations and insulin concentrations and between BMI and insulin concentrations, patients were stratified by presence of 25(OH)D deficiency (defined as less than 75 nmol/L) and by presence of obesity (BMI ≥ 30 kg/m²). Mean fasting insulin concentrations and confidence intervals were assessed and compared among multiple groups using the all-pairs Tukey-Kramer test. Then we used multiple regression analysis to evaluate the relationship between fasting insulin concentrations and the predictors of interest. In order to characterize the independent associations between 25(OH)D concentrations, BMI category, AD treatment and the fasting insulin outcome, each of the predictors was sequentially added into the multivariate regression models. In Model I, we regressed fasting insulin on BMI and 25(OH)D, Model II additionally incorporated the dichotomous AD treatment variable, and Model III included all three variables plus an interaction term between AD treatment and BMI category. The interaction term was derived from the product of dummy variables (1,0) for each variable. Age, gender, race, parathyroid hormone (PTH), estimated Glomerular Filtration rate (eGFR), and systolic blood pressure (SBP) covariates were then included into the final, fully adjusted model (Model IV). Because changes in calcium and phosphorus concentrations directly affected PTH concentrations, they were not included in the multivariate analysis. We did not transform data in order to preserve the clinical interpretability of the results. However, separate analysis with transformation of variables that were not normally distributed did not substantively alter the results.

All analyses were performed using JMP 8, a component of SAS(S. Carey, NC).

Results

As seen in Table I, the mean fasting insulin level was 48.5 ± 28.4 pmol/L and the mean 25(OH)D level was 71.4 ± 29.2 nmol/L. Mean fasting insulin concentrations did not differ significantly by gender (female 45.0 ± 28.6 vs men 50.3 ± 28.2 pmol/L, $P=0.35$), and had non-significant correlations with age ($r=-0.10$, $P=0.27$), eGFR ($r=0.06$, $P=0.5$), systolic blood pressure ($r=0.13$, $P=0.14$) and parathyroid hormone ($r=0.14$, $P=0.13$). 25(OH)D concentrations were significantly associated with PTH concentrations ($r=-0.34$, $P=0.0004$),

but not with calcium ($r=0.15$, $P=0.12$) or phosphorus ($r=-0.12$, $P=0.23$) concentrations. 25(OH)D concentrations also varied indirectly with BMI ($r=-0.38$, $P<.0001$).

Compared to patients not receiving AD, patients being treated with AD were older (66.0 ± 16.7 vs 58.7 ± 16.3 yrs, $P=0.02$), had worse renal function (eGFR 24.0 ± 14.0 vs 43.8 ± 24.0 ml/min, $P<.0001$), and had higher serum phosphate and lower serum calcium concentrations, but otherwise had similar blood pressures, 25(OH)D concentrations, and BMIs. AD treated patients had significantly lower fasting insulin concentrations than those not receiving AD (40.5 ± 22.0 vs 54.1 ± 30.9 pmol/L, $P=0.01$). Except for four patients who were treated with oral doxercalciferol (mean dose of 2.8 ± 1.4 mcg/week), all patients in the AD group were receiving oral calcitriol, at a mean dose of 1.7 ± 1.3 mcg/week. 43 patients (22 in the AD group) were currently taking vitamin D supplementation, primarily in the form of over-the-counter supplementation (dose range of 400 to 800 IU). Five patients were taking 50,000 IU of prescribed ergocalciferol weekly (3 in the AD group.)

Mean fasting insulin level correlated significantly and negatively with both 25(OH)D concentrations ($r=-0.22$, $P=0.02$) and BMI ($r=-0.36$, $P<.0001$). 25(OH)D concentrations varied negatively with BMI ($r=-0.38$, $P<.0001$). Univariate analysis of 25(OH)D and fasting insulin (Figure 1) showed a moderate association between 25(OH)D and fasting insulin concentrations ($\beta_{25\text{OHD}} = -0.21$, $P=0.02$) in the whole cohort. As seen in figure II, in those being treated with AD, the association between 25(OH)D concentrations and fasting insulin was greatly attenuated ($r=0.05$, $P=0.73$), whereas for those who were AD naive, the linear association was strengthened ($r=-0.37$, $P=0.002$). The duration of previous AD treatment is not known. Inclusion of eGFR in multivariate analysis did not significantly alter the associations (data not shown.)

Patients were then stratified by the presence of 25(OH)D deficiency, as defined by a 25(OH)D concentration of 75.0 nmol/L, and AD treatment (Figure III.) For those with sufficient 25(OH)D concentrations, treatment with AD was not associated with a difference in mean fasting insulin concentrations (41.6 ± 9.9 vs 41.4 ± 7.7 pmol/L, $P=0.98$ (treated vs untreated). However, in 25(OH)D deficient patients, replacement with AD was associated with significantly lower insulin concentrations than the untreated group (40.5 ± 9.2 vs 63.9 ± 10.9 pmol/L, $P=0.003$). There were insignificant differences in age ($P=0.15$) and systolic blood pressure ($P=0.24$) across the groups; the AD group in each 25(OH)D category had similar BMIs to the AD naive group but significantly lower renal function. The eGFR in the AD treated versus untreated group was 24.2 ± 5.8 vs 42.5 ± 5.06 ml/min ($P<.0001$) in those with 25(OH)D concentrations less than 75.0 nmol/L, and 26.8 ± 7.7 vs 45.6 ± 5.4 ml/min ($P=0.0007$) in those who were 25(OH)D replete.

Given the close correlation between 25(OH)D deficiency and obesity, it is plausible that any observed relationship between 25(OH)D concentrations and fasting insulin is due to a confounding effect of BMI. In order to investigate the independent effect of 25(OH)D, BMI, and AD treatment on insulin resistance, multiple regression analyses were performed, sequentially adding BMI (Model I) and treatment with AD (Model II) to the baseline univariate association between 25(OH)D and fasting insulin, as seen in Table II. The inclusion of BMI (Model I) greatly weakened the association between 25(OH)D concentrations and fasting insulin. As seen in model II, both BMI and AD treatment contributed independently to the variation of the fasting insulin concentrations. A borderline significant interaction term included in a final, fully adjusted model (Model IV) ($P=0.03$) suggests that the effect of treatment with AD varies with the presence of obesity.

To further investigate how AD treatment affects the association between obesity and insulin resistance, patients were stratified by BMI and treatment with AD (Figure IV). As expected,

the patients in the lower BMI category had low mean fasting insulin concentrations (95% CI's), which did not differ by whether they were receiving AD or not (37.9 ± 7.8 vs 41.7 ± 6.6 pmol/L, $P=0.91$). However, for those patients in the higher BMI category, treatment with AD was associated with significantly lower insulin concentrations ($P=0.003$). Those who were not receiving AD had a mean fasting insulin level of 74.4 ± 13.6 pmol/L, while those on AD had a mean level of 46.1 ± 13.3 pmol/L, similar to individuals in the lower BMI category.

Conclusions

Our findings add to a growing literature supporting a critical role of vitamin D in insulin sensitivity and metabolism. In our analysis, 25(OH)D concentrations were inversely associated with fasting insulin concentrations in patients with nondiabetic CKD, and treatment with AD was associated with lower insulin concentrations.

Given the cross-sectional nature of this study, a causal relationship between vitamin D and insulin resistance cannot be established. However, evaluating how the administration of AD alters the relationship between 25(OH)D and fasting insulin concentrations affords the opportunity to consider the biologic basis of the association. If vitamin D deficiency causes insulin resistance in CKD, then replacement with the biologically active metabolite, bypassing any defect in renal 1-alpha hydroxylase activity, should override any association between 25(OH)D and insulin concentrations. Indeed, in our analysis, the inverse association between 25(OH)D and fasting insulin concentrations was eliminated in those on AD, and strengthened in those not receiving AD.

Furthermore, our data indicates that AD replacement may improve insulin sensitivity in vitamin D deficient patients. In patients with 25(OH)D concentrations less than 75 nmol/L, treatment with AD was associated with lower insulin concentrations than the untreated group, despite those patients having worse renal function and more severe secondary hyperparathyroidism, both of which have been associated with insulin resistance(12). Interestingly, 25(OH)D deficient patients treated with AD had similar fasting insulin concentrations to 25(OH)D replete patients, suggesting that there is a biologic threshold for the effect of vitamin D on insulin metabolism and signaling.

Small studies have suggested that AD treatment improves insulin sensitivity in dialysis patients(13–14), yet has not been studied in the predialysis CKD population where renal 1-alpha hydroxylase activity is harder to predict. AD treatment has been linked to improved outcomes in patients with renal disease, including reduced mortality(15). Among the myriad potential mechanistic explanations for this observed phenomenon are benefits on vascular function(16–17), blood pressure(18) and left ventricular hypertrophy(19–20), but may include a beneficial effect on either insulin secretion, possibly by modulating beta cell function(21–24), or on insulin sensitivity.

Our findings challenge the assumption that CKD patients cannot metabolize 25(OH)D into 1,25(OH)D. Patients who were 25(OH)D replete had low fasting insulin concentrations despite a wide range of renal dysfunction. This raises the possibility that either residual renal 1-alpha hydroxylase persists despite significant loss of eGFR(25), or that extra-renal hydroxylation facilitates the conversion of 25(OH)D to active vitamin D within insulin responsive sites.

Given the possibility of extra-renal hydroxylation of 25(OH)D, it is plausible that either cholecalciferol or ergocalciferol supplementation might provide the same benefits for cardiovascular disease as has been seen with AD in CKD. 25(OH)D sufficiency has been linked to lower mortality than 25(OH)D deficiency in CKD(26) and dialysis patients(27),

independently of AD treatment, supporting the biologic importance of 25(OH)D in this population. Given the increased risk of hypercalcemia associated with AD treatment compared to ergocalciferol, a well designed study comparing ergocalciferol to AD treatment in patients with predialysis CKD would be useful. No study has evaluated whether ergocalciferol supplementation might provide the same benefit on insulin metabolism as AD in patients with CKD.

An association between obesity and vitamin D deficiency potentially accounts for part of the relationship between obesity and insulin resistance(28–29). Obese individuals have lower 25(OH)D concentrations, higher PTH concentrations(30), and lower 1,25(OH)D concentrations than nonobese individuals(31). In addition, adiposity prevents the beneficial response to dietary vitamin D supplementation (32). In our analysis, both AD treatment and BMI were independent predictors of insulin resistance. However, when stratified by BMI, treatment with AD in obese patients was associated with significantly lower fasting insulin concentrations than obese patients who were not receiving AD. In the subgroup with low BMI, treatment with AD was not associated with a difference in fasting insulin concentrations, likely accounted for by less 25(OH)D deficiency than in the obese group. These findings suggest that vitamin D deficiency might be a remediable risk factor for insulin resistance in obesity. Given that adipose tissue might act as a biologic sink for fat soluble vitamins(33), preventing the storage form from being converted into the biologically active form, the administration of activated D compounds, which are more polar in nature and thus not drawn into fat tissue, may be more efficacious than ergocalciferol in obese patients. No study to our knowledge has compared the effect of ergocalciferol to AD in obese patients.

The modifying effect of AD on the relationship between 25(OH)D and fasting insulin, and the observation that replacement with AD is associated with lower fasting insulin concentrations, support a causal role of vitamin D deficiency in insulin resistance. However, the observational design of this study prohibits definitive conclusions regarding causality, and further well designed studies are necessary to better elucidate the relationship between vitamin D status and insulin resistance.

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References

1. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care*. 2004 Dec; 27(12):2813–8. [PubMed: 15562190]
2. Ford ES, Zhao G, Li C, Pearson WS. Serum concentrations of vitamin D and parathyroid hormone and prevalent metabolic syndrome among adults in the United States. *J Diabetes*. 2009 Dec; 1(4): 296–303. [PubMed: 20923530]
3. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract*. 1995 Mar; 27(3):181–8. [PubMed: 7555599]
4. Nagpal J, Pande JN, Bhartia A. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. *Diabet Med*. 2009 Jan; 26(1):19–27. [PubMed: 19125756]
5. Tai K, Need AG, Horowitz M, Chapman IM. Glucose tolerance and vitamin D: effects of treating vitamin D deficiency. *Nutrition*. 2008 Oct; 24(10):950–6. [PubMed: 18653316]

6. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr.* 2010 Feb; 103(4):549–55. [PubMed: 19781131]
7. Need AG, O'Loughlin PD, Horowitz M, Nordin BE. Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. *Clin Endocrinol (Oxf).* 2005 Jun; 62(6):738–41. [PubMed: 15943837]
8. McGill AT, Stewart JM, Lithander FE, Strik CM, Poppitt SD. Relationships of low serum vitamin D3 with anthropometry and markers of the metabolic syndrome and diabetes in overweight and obesity. *Nutr J.* 2008; 7:4. [PubMed: 18226257]
9. Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care.* 2005 May; 28(5):1228–30. [PubMed: 15855599]
10. http://www.kidney.org/professionals/KDOQI/guidelines_bone/Guide7.htm.
11. http://www.kidney.org/professionals/KDOQI/guidelines_bone/Guide8A.
12. Massry SG, Fadda GZ. Chronic renal failure and secondary hyperparathyroidism are responsible for glucose intolerance and impaired insulin secretion of aging. *J Endocrinol Invest.* 1992; 15(9 Suppl 6):73–7. [PubMed: 1300343]
13. Turk S, Yeksan M, Tamer N, Gurbilek M, Erdogan Y, Erkul I. Effect of 1,25 (OH)2D3 treatment on glucose intolerance in uraemia. *Nephrol Dial Transplant.* 1992; 7(12):1207–12. [PubMed: 1337161]
14. Mak RH. Intravenous 1,25 dihydroxycholecalciferol corrects glucose intolerance in hemodialysis patients. *Kidney Int.* 1992 Apr; 41(4):1049–54. [PubMed: 1513086]
15. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med.* 2003 Jul 31; 349(5):446–56. [PubMed: 12890843]
16. Talmor Y, Bernheim J, Klein O, Green J, Rashid G. Calcitriol blunts pro-atherosclerotic parameters through NFkappaB and p38 in vitro. *Eur J Clin Invest.* 2008 Aug; 38(8):548–54. [PubMed: 18717824]
17. Mathew S, Lund RJ, Chaudhary LR, Geurs T, Hruska KA. Vitamin D receptor activators can protect against vascular calcification. *J Am Soc Nephrol.* 2008 Aug; 19(8):1509–19. [PubMed: 18448587]
18. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* 2002 Jul; 110(2):229–38. [PubMed: 12122115]
19. Bodyak N, Ayus JC, Achinger S, Shivalingappa V, Ke Q, Chen YS, Rigor DL, Stillman I, Tamez H, Kroeger PE, Wu-Wong RR, Karumanchi SA, Thadhani R, Kang PM. Activated vitamin D attenuates left ventricular abnormalities induced by dietary sodium in Dahl salt-sensitive animals. *Proc Natl Acad Sci U S A.* 2007 Oct 23; 104(43):16810–5. [PubMed: 17942703]
20. Kong J, Kim GH, Wei M, Sun T, Li G, Liu SQ, Li X, Bhan I, Zhao Q, Thadhani R, Li YC. Therapeutic effects of vitamin D analogs on cardiac hypertrophy in spontaneously hypertensive rats. *Am J Pathol.* 2010 Aug; 177(2):622–31. [PubMed: 20616348]
21. Kayaniyl S, Vieth R, Retnakaran R, Knight JA, Qi Y, Gerstein HC, Perkins BA, Harris SB, Zinman B, Hanley AJ. Association of vitamin D with insulin resistance and beta-cell dysfunction in subjects at risk for type 2 diabetes. *Diabetes Care.* 2010 Jun; 33(6):1379–81. [PubMed: 20215450]
22. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. *Diabetes.* 2008 Oct; 57(10):2619–25. [PubMed: 18591391]
23. Mak RH, Wong JH. The vitamin D/parathyroid hormone axis in the pathogenesis of hypertension and insulin resistance in uremia. *Miner Electrolyte Metab.* 1992; 18(2–5):156–9. [PubMed: 1465050]
24. Labriji-Mestaghanmi H, Billaudel B, Garnier PE, Malaisse WJ, Sutter BC. Vitamin D and pancreatic islet function. I. Time course for changes in insulin secretion and content during vitamin D deprivation and repletion. *J Endocrinol Invest.* 1988 Sep; 11(8):577–84. [PubMed: 3072373]

25. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C. Evidence for persistent vitamin D 1-alpha-hydroxylation in hemodialysis patients: evolution of serum 1,25-dihydroxycholecalciferol after 6 months of 25-hydroxycholecalciferol treatment. *Nephron Clin Pract.* 2008; 110(1):c58–65. [PubMed: 18724068]
26. Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, Mallamaci F, Zoccali C. Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int.* 2009 Jan; 75(1):88–95. [PubMed: 18843258]
27. Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA Jr, Tonelli M, Thadhani R. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int.* 2007 Oct; 72(8):1004–13. [PubMed: 17687259]
28. Renzaho AM, Halliday JA, Nowson C. Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: A systematic review. *Nutrition.* 2011 Sep; 27(9):868–79. [PubMed: 21704500]
29. Hypponen E, Power C. Vitamin D status and glucose homeostasis in the 1958 British birth cohort: the role of obesity. *Diabetes Care.* 2006 Oct; 29(10):2244–6. [PubMed: 17003300]
30. Grethen E, McClintock R, Gupta CE, Jones R, Cacucci BM, Diaz D, Fulford AD, Perkins SM, Considine RV, Peacock M. Vitamin D and Hyperparathyroidism in Obesity. *J Clin Endocrinol Metab.* 2011 Feb 16.
31. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab.* 2004 Mar; 89(3):1196–9. [PubMed: 15001609]
32. Dong Y, Stallmann-Jorgensen IS, Pollock NK, Harris RA, Keeton D, Huang Y, Li K, Bassali R, Guo DH, Thomas J, Pierce GL, White J, Holick MF, Zhu H. A 16-week randomized clinical trial of 2000 international units daily vitamin D3 supplementation in black youth: 25-hydroxyvitamin D, adiposity, and arterial stiffness. *J Clin Endocrinol Metab.* 2010 Oct; 95(10):4584–91. [PubMed: 20660028]
33. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr.* 2000 Sep; 72(3):690–3. [PubMed: 10966885]

Figure 1. Univariate association between 25(OH)D and fasting insulin concentrations ($r=-0.22$, $\beta_{25\text{OHD}} = -0.21$, $P=0.02$).

Figure II. Association between 25(OH)D and fasting insulin concentrations in those receiving (a) and not receiving (b) activated vitamin D

Treatment with AD greatly weakened the association between 25(OH)D and fasting insulin to nonsignificance ($r = 0.05$, $\beta_{25\text{OHD}} = 0.04$, $P = 0.73$), whereas those not receiving AD, the association was strengthened ($r = -0.37$, $\beta_{25\text{OHD}} = -0.36$, $P = 0.002$)

Figure III. Mean fasting insulin concentrations stratified by 25(OH)D concentrations (nmol/L) and treatment with AD
95% confidence intervals provided.

Figure IV. Mean fasting insulin level stratified by BMI (kg/m^2) and treatment with AD 95% confidence intervals provided.

Table 1

Baseline characteristics of study population.

	Total population (n=120)	+ AD (n=49)	– AD (n=71)	P value
Age (yrs)	61.7 ± 16.7	66.0 ± 16.7	58.7 ± 16.3	0.02
Gender (%female)	32.5%	32.7%	32.4%	0.97
Race				
% White	70.2%	65.3%	73.2%	0.32
% Black	19.2%	26.5%	14.1%	
% Asian	9.2%	6.1%	11.3%	
% Hispanic	1.6%	2.0%	1.4%	
BMI(kg/m ²)	28.7 ± 7.1	28.5 ± 6.5	28.7 ± 7.5	0.85
SBP (mm/Hg)	127.7 ± 16.0	128.4 ± 14.7	127.4 ± 17.0	0.71
DBP (mm/Hg)	73.5 ± 10.2	71.5 ± 10.5	74.9 ± 9.9	0.08
Creatinine (μmol/L)	176.8 ± 114.8	229.8 ± 132.6	141.4 ± 79.5	<.0001
eGFR (ml/min)	32.0 ± 26.0	24.0 ± 14.0	43.8 ± 24.0	<.0001
Calcium (nmol/L)	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.1	0.02
Phosphate (nmol/L)	1.2 ± 0.3	1.3 ± 0.4	1.1 ± 0.2	0.0005
PTH (pg/mL)	80.0 ± 74.8	104.0 ± 69.0	96.2 ± 73.0	0.06
25(OH)D (nmol/L)	71.4 ± 29.2	68.1 ± 25.7	73.6 ± 31.2	0.32
Fasting Insulin (pmol/L)	48.5 ± 28.4	40.5 ± 22.0	54.1 ± 30.9	0.01

Means ± standard deviation for all variables, except creatinine, eGFR and PTH (medians ± inter quartile range). P values represent significance of differences between AD treatment groups. +AD indicates patients receiving AD, whereas –AD indicates patients not receiving AD. BMI = body mass index, SBP = systolic blood pressure, BP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, PTH = parathyroid hormone. To convert creatinine from μmol/l to mg/dl, divide by 88.4; calcium from mmol/L to mg/dl to, divide by 0.25; phosphorus from mmol/L to mg/dL, divide by 0.323; 25(OH)D from nmol/L to ng/ml, divide by 2.496; and insulin from pmol/L to uU/mL to, divide by 6.95.

Table II
Multivariate analysis of the association between 25(OH)D, BMI category, and AD treatment with fasting insulin

Beta coefficients $\pm$ standard errors and (p-values) are provided for each variable. Model I, II, and III reflect stepwise addition of BMI category, AD treatment, and an interaction term, respectively, to the univariate analysis between 25(OH)D and fasting insulin. Model IV adds age, gender, race, PTH, eGFR, and systolic blood pressure to create the final, fully adjusted model.

	Fasting Insulin Level (pmol/L)			
	Model I $R^2 = 0.16$ $P < .0001$	Model II $R^2 = 0.22$ $P < .0001$	Model III $R^2 = 0.26$ $P < .0001$	Model IV $R^2 = 0.35$ $P < .0001$
25(OH)D (nmol/L)	$-0.08 \pm 0.09(0.39)$	$-0.1 \pm 0.09(0.24)$	$-0.06 \pm 0.09(0.52)$	$-0.05 \pm 0.1(0.64)$
BMI (< 30)	$21.1 \pm 5.5(0.0002)$	$20.7 \pm 5.3(0.0002)$	$19.7 \pm 5.2(0.0002)$	$24.1 \pm 5.7(<.0001)$
AD (+)	-	$-7.3 \pm 2.4(0.003)$	$-2.8 \pm 3.0(0.36)$	$-0.49 \pm 3.5(0.88)$
Interaction term BMI (< 30) and AD(+)	-	-	$-12.1 \pm 5.0(0.02)$	$-11.6 \pm 5.3(0.03)$