Preferential Vascular Deposition of Tissue Phosphate Linked to Impaired Phosphate Kinetics Leads to Calcification in Experimental Chronic Kidney Disease

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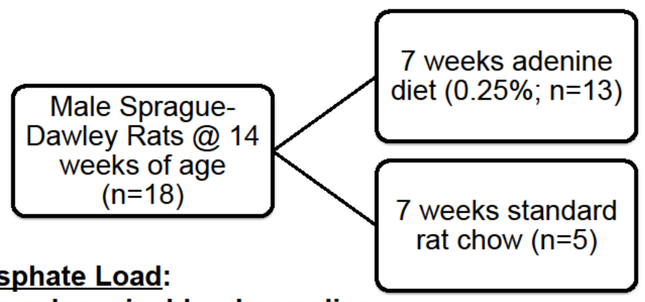
Introduction

Background: Pathogenic vascular calcification (VC), a process linked to altered phosphate metabolism is a marker of advancing CVD in CKD patients.[1] Isakova *et* al (2008) compared acute post-prandial phosphate metabolism in healthy individuals and in patients with moderate CKD.[2] There was a marked and significant increase in the urinary appearance of phosphate in healthy individuals by 30 minutes. In contrast, serum phosphate and the urine fractional excretion of phosphate did not change after ingesting a high phosphate meal (500mg) in patients with CKD. The maintenance of normal phosphate levels in the blood in the absence of phosphate in the urine strongly suggests that extra-renal phosphate disposition occurs in CKD. However, the overall tissue disposition of phosphate acutely *in vivo* is currently unknown.

Purpose: The study objective was to characterize the changes in phosphate tissue distribution that could underlie the vascular pathogenesis seen in experimental CKD.

Experimental Design



Intravenous Phosphate Load:

- **Cannulate the jugular vein, blood sampling from contralateral jugular vein**
- **3mL of 100mM phosphate (p-33, 100uC) at constant infusion rate (LCS analysis).**

Figure 1 Experimental Design. Male Sprague-Dawley rats were maintained on a CKD generating diet (n=13; 0.25% adenine) for 7 weeks.

Blood Parameters	Controls	CKD (VC-)	CKD (VC+)
Cr (uM)	26.8 ± 8	329.3 ± 186 *	375.2 ± 59 *
PO_4^{2-} (mM)	1.5 ± 0.3	5.1 ± 1.8 *	5.9 ± 1.4 *
Ca^{2+} (mM)	2.5 ± 0.4	2.5 ± 0.4	2.6 ± 0.5
Mg^{2+} (mM)	1.2 ± 0.3	1.5 ± 0.5	1.5 ± 0.4
PTH (pg/mL)	357.9 ± 210	3453 ± 1646 *	7576 ± 3325 #
FGF 23 (pg/mL)	235.6 ± 52	15943.8 ± 11131 *	25064.0 ± 13969 *

Figure 2 Serum and plasma parameters in control and adenine (7wk CKD; 0.25%) fed rats. Data are expressed mean ± SD. Serum: Cr, creatinine; PO_4^{2-}, phosphate; Ca^{2+}, calcium; Plasma: PTH, parathyroid horomone; FGF 23, fibroblast growth factor 23. CKD rats were divided into two groups based on the presence (n=6) or absence (n=7) of vascular calcification. *p<0.05 significantly different than control. #p<0.05 significantly different than controls and CKD (VC-).

Results

Mineral Kinetics

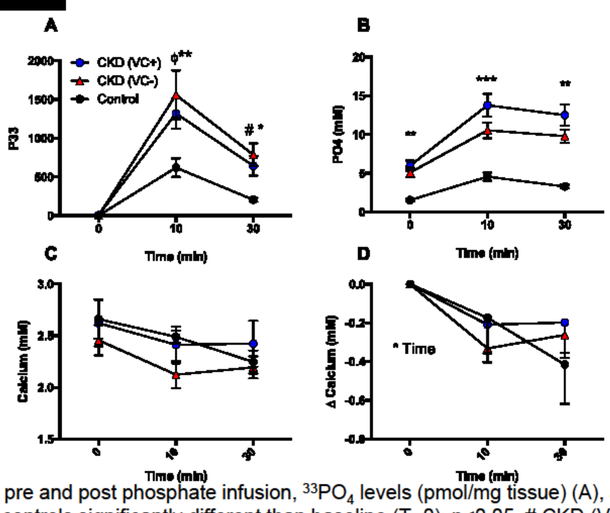


Figure 3 Serum mineral levels pre and post phosphate infusion, $^{33}PO_4$ levels (pmol/mg tissue) (A), and total PO_4 levels (B). Φ CKD (VC+), CKD (VC-) and controls significantly different than baseline (T=0), p<0.05. # CKD (VC-) and CKD (VC+) significantly different than baseline, p<0.05. *CKD (VC-) significantly different than control, p<0.05. ** CKD (VC-) and CKD (VC+) significantly different than controls, p<0.05. *** All groups significantly different from each other, p<0.05 .

Δ Serum Proteins

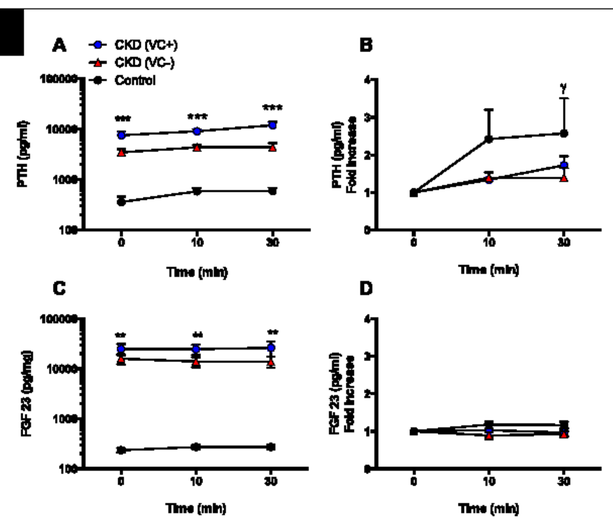


Figure 4 Plasma PTH and FGF 23 levels post phosphate infusion. ** CKD VC+ and VC- significantly different than CON, p<0.05. ***All groups significantly different from each other, p<0.05 . γ Fold increase in control greater than CKD VC+ and VC-, p<0.05.

Tissue $^{33}PO_4$ Distribution

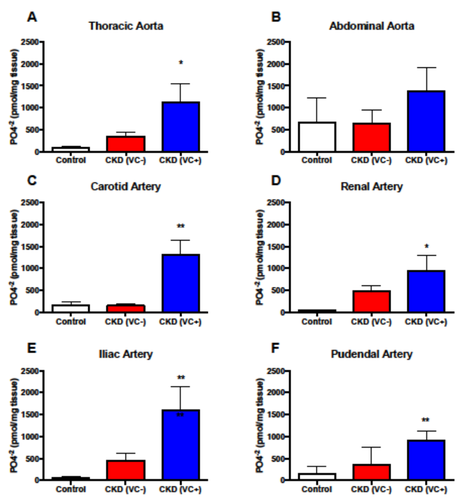


Figure 7 *In vivo* $^{33}PO_4$ distribution post infusion in various vascular beds. * significantly different than controls, **significantly different than controls and CKD (VC-), p<0.05.

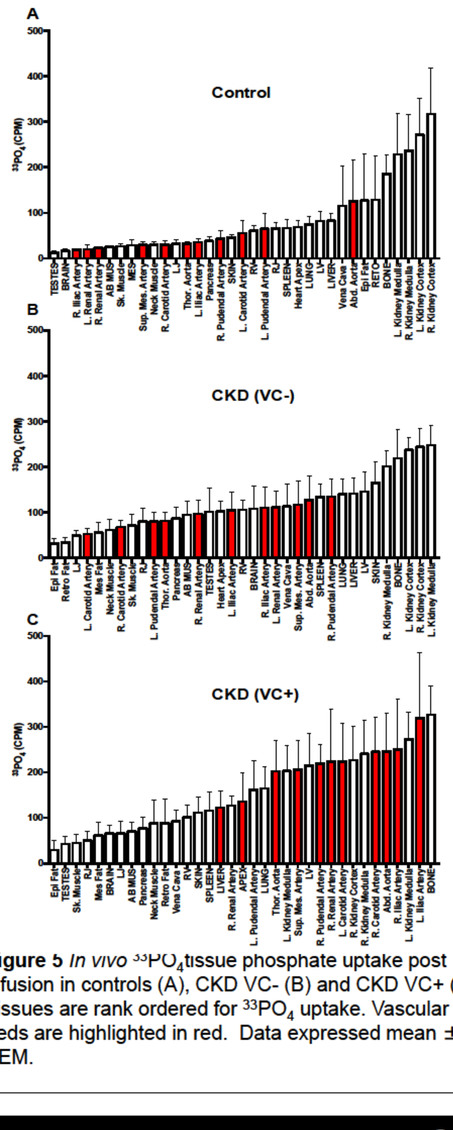


Figure 5 *In vivo* $^{33}PO_4$ tissue phosphate uptake post infusion in controls (A), CKD VC- (B) and CKD VC+ (C). Tissues are rank ordered for $^{33}PO_4$ uptake. Vascular beds are highlighted in red. Data expressed mean ± SEM.

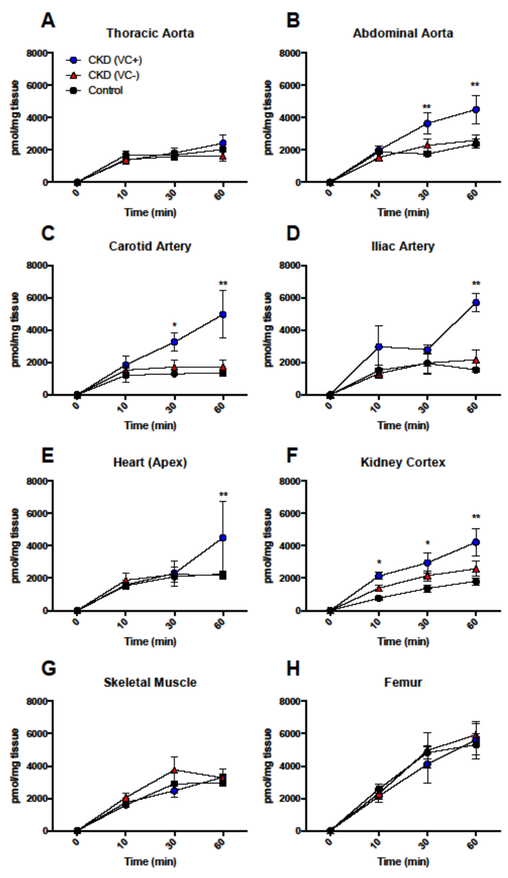


Figure 6 *Ex vivo* phosphate kinetics measured with p33 in the thoracic aorta (A), abdominal aorta (B), carotid artery (C), Iliac artery (D), apex of the heart (E), kidney cortex (F), quadriceps skeletal muscle (G), and femur bone (H). Tissue segments were incubated in calcification media with 3.8mM phosphate+p33. * Significantly different than control, **significantly different than control and CKD (VC-), p<0.05.

Conclusions

1) The Disposition of circulating phosphate is dramitically altered in CKD, particularly following the onset of pathogenic VC.
2) The progressive and preferential deposition of phosphate into vascular tissue in CKD provides compelling evidence that phosphate dysregulation at the level of each tissue underlies the development of calcification.

References

1. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis. 1998;32(5 Suppl 3):S112-9.
2. Isakova T, Gutierrez O, Shah A, et al. Postprandial mineral metabolism and secondary hyperparathyroidism in early CKD. J Am Soc Nephrol. 2008;19(3):615-623.

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