Vascular Physiology: Changes Associated with Vit D

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8/4/09 - 11:10-11:25AM present

11:25-11:40 discussion

Objectives

- Relate vascular calcification in our CKD translational model to the wider issue of atherosclerotic cardiovascular risk as affected by vascular calcification.
- Describe the effects of vitamin D analogs on vascular calcification in CKD and beyond.

Vitamin D Use In Chronic Kidney Disease (CKD)

- CKD is the number one cardiovascular risk factor worldwide.
- Cardiovascular mortality in CKD is excessive (a 30yo on dialysis has the life expectancy of an 80yo with nl kid function)
- Vascular calcification (VC) in CKD is one of the causes of the excess cardiovascular risk
- Vitamin D analogs are widely used in CKD and high doses/intoxication causes VC

Vitamin D Receptor Activation (VDRA) in CKD

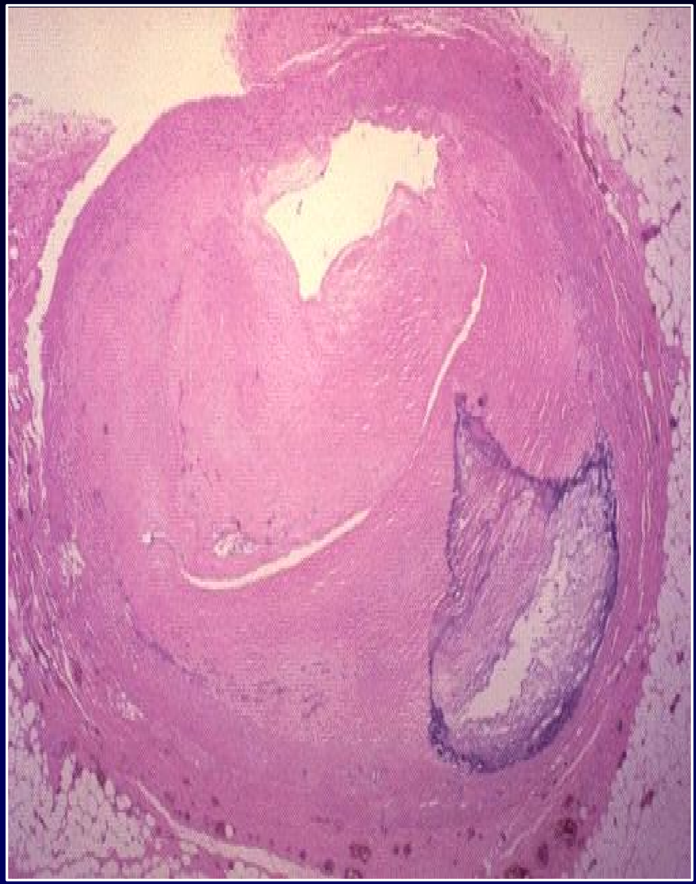
- Vitamin D analogs are approved drugs for the treatment of secondary hyperparathyroidism in CKD, and large doses of vitamin D receptor activators are used in dialysis patients.
- Several large observational studies demonstrate survival advantage in ESKD patients receiving VDRAs.
- Mechanism of VDRA action in increasing patient survival is unknown

Translational Studies Demonstrate Vitamin D Analogs Stimulate VC

- Wu Wong et al, comparison of doxercalciferol to paricalcitol
- Slatopolsky laboratory, doxercalciferol comparison to paricalcitol
- Cardus et al, JBMR 2007, compared calcitriol to paricalcitol
- Studies in the 5/6 NPX rat model fed high PO4 to induce VC.

Vascular Calcification (VC) in CKD

Atherosclerotic	Mönkeberg
Arterial Intimal Calcification	Arterial Medial Calcification



- In CKD both forms of VC are observed in the large arteries, calciphylaxis is mainly Mönkeberg, coronary artery calcification and cardiac valve calcification are atherosclerotic

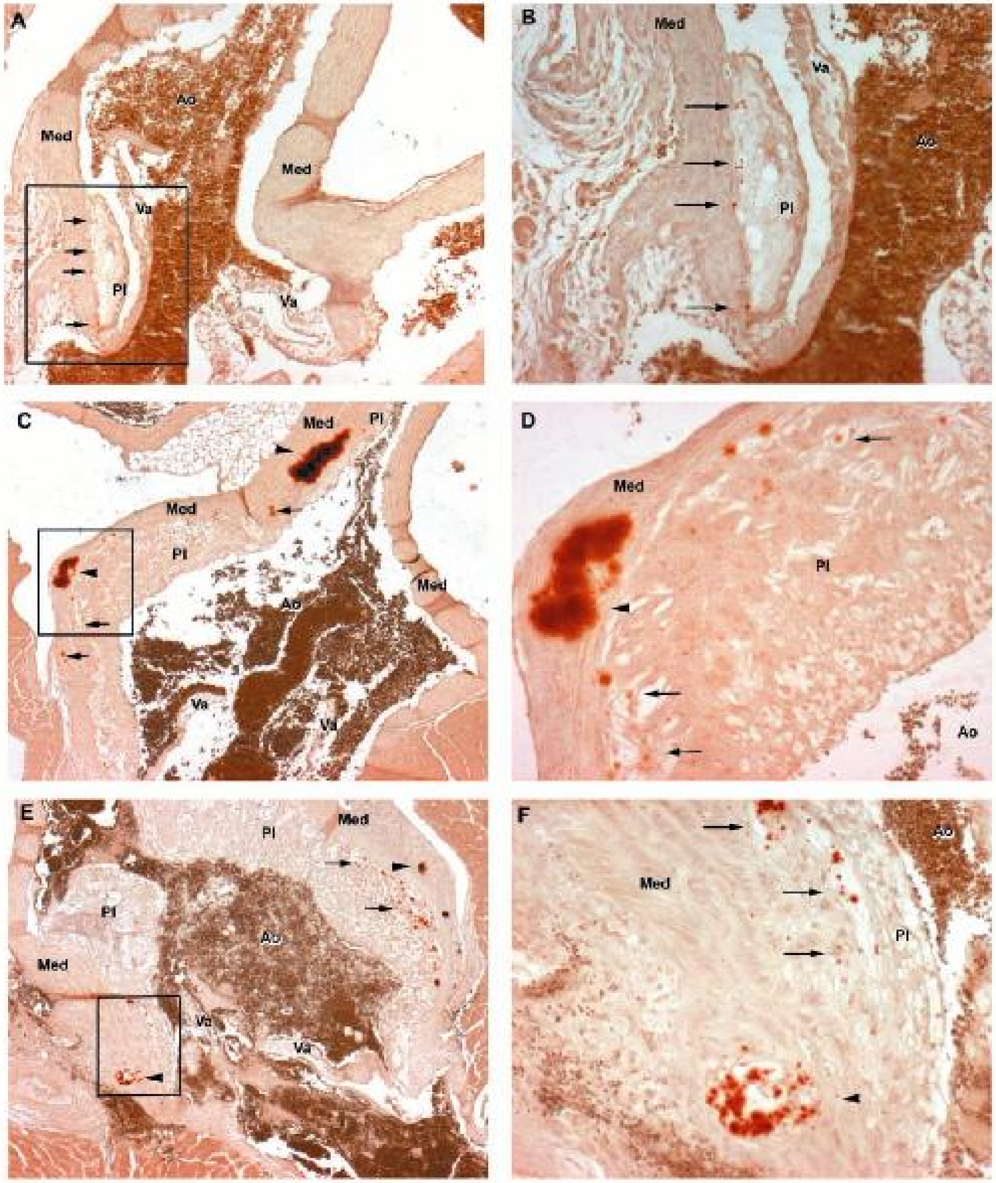
Translational Studies Demonstrate Vit D Analogs Are Protective Against VC

- Mathews et al, JASN 2008 demonstrate calcitriol and paricalcitol are protective at lose doses against VC (data to follow)
- Mathews et al, ASN 2007-9 showing doxercalciferol is protective and discovers mechanism of protection VC (data to follow)

Methods

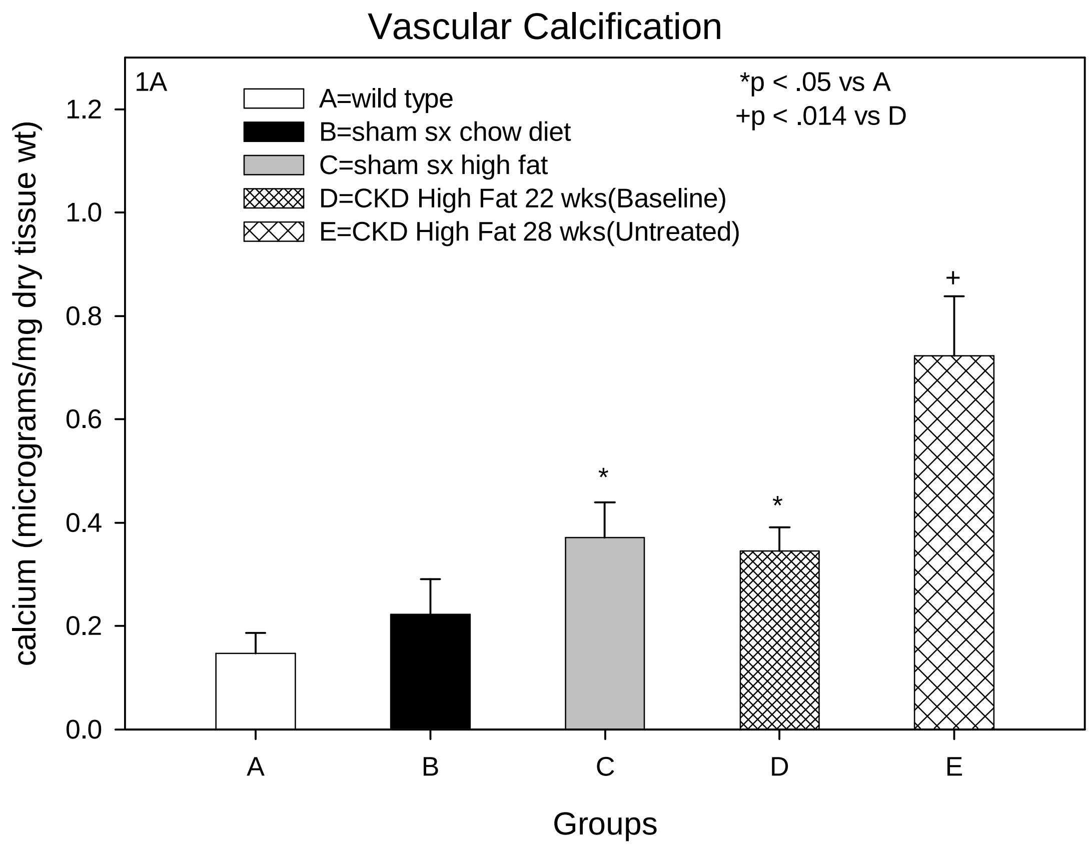
- LDLR-/- mice (familial hypercholesterolemia) fed a high fat western diet beginning at 10 wks. CKD induced by renal ablation at 12 wks. At 22 weeks when vascular calcification was well established, treatment was begun with vit D analogs.
- Model of atherosclerosis, insulin resistance type 2 diabetes and DM/CKD

Effect of Uremia and Diet on Vascular Calcification in the LDLR Null mouse



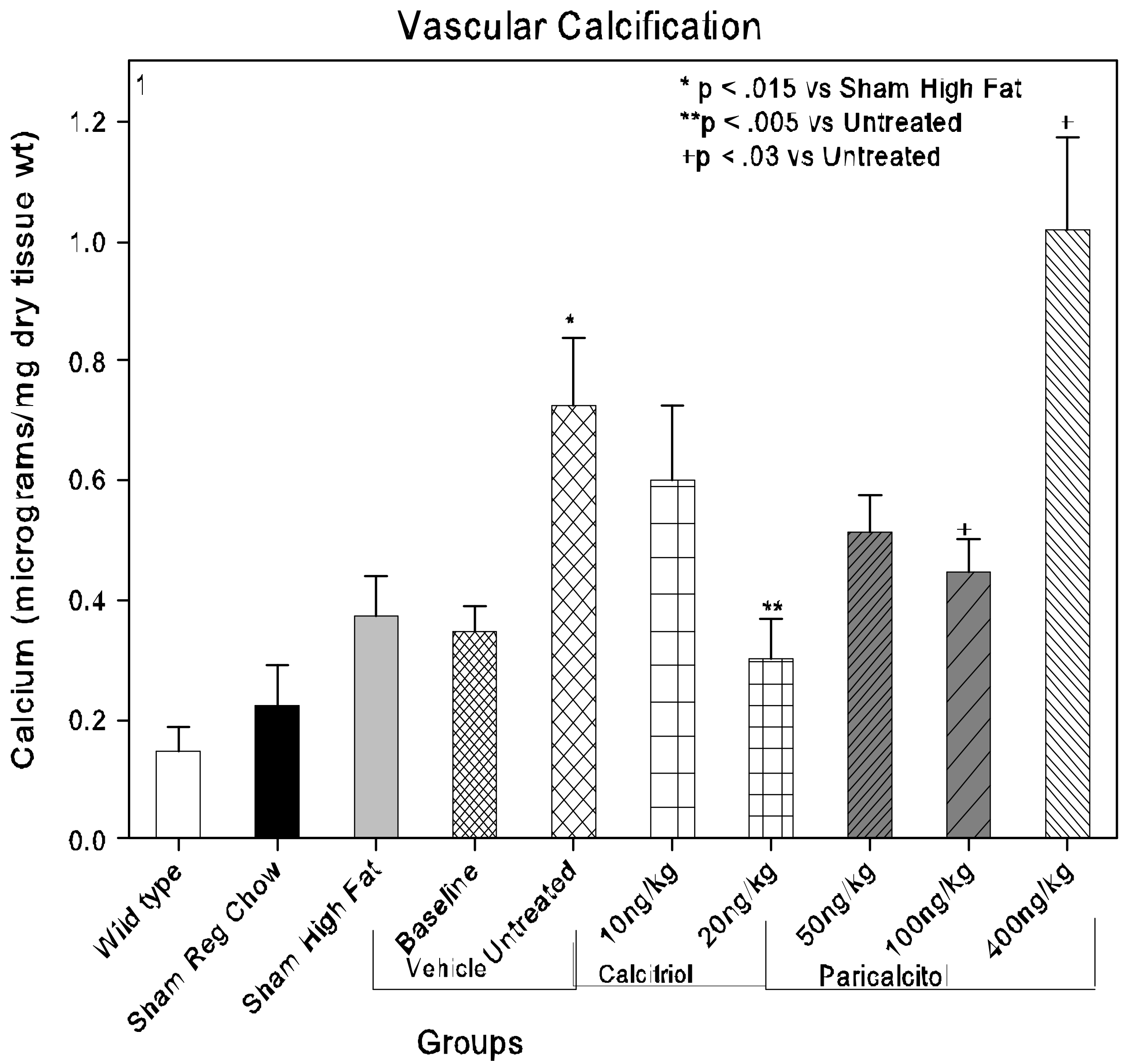
Davies et al
JASN, 2003

Vascular Calcification in a translational animal model of atherosclerosis, the LDLR-/- high fat fed mouse with ablative CKD



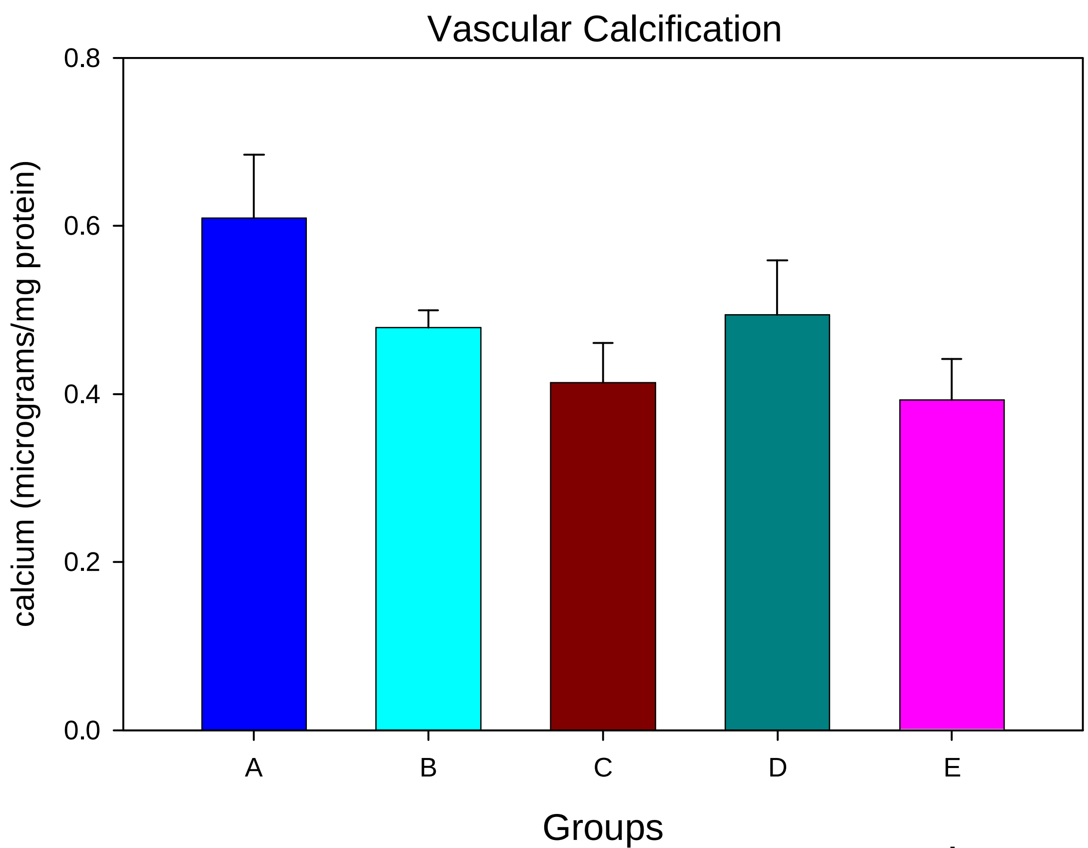
Protocol

- Beginning at 22 wks, going to 28 wks
  - 10 or 20ng/kg of calcitriol IP TIW
  - 50, 100, or 400 ng/kg of paricalcitol IP TIW
- Measure aortic Ca, bone histomorph.



Vascular Calcification
1
* p < .015 vs Sham High Fat
**p < .005 vs Untreated
+p < .03 vs Untreated
Calcium (micrograms/mg dry tissue wt)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Wild type
Sham Reg Chow
Sham High Fat
Baseline
Untreated
Vehicle
10ng/kg
20ng/kg
Calcitriol
50ng/kg
100ng/kg
400ng/kg
Paricalcitol
Groups

Doxercalciferol / Sevelamer Carbonate



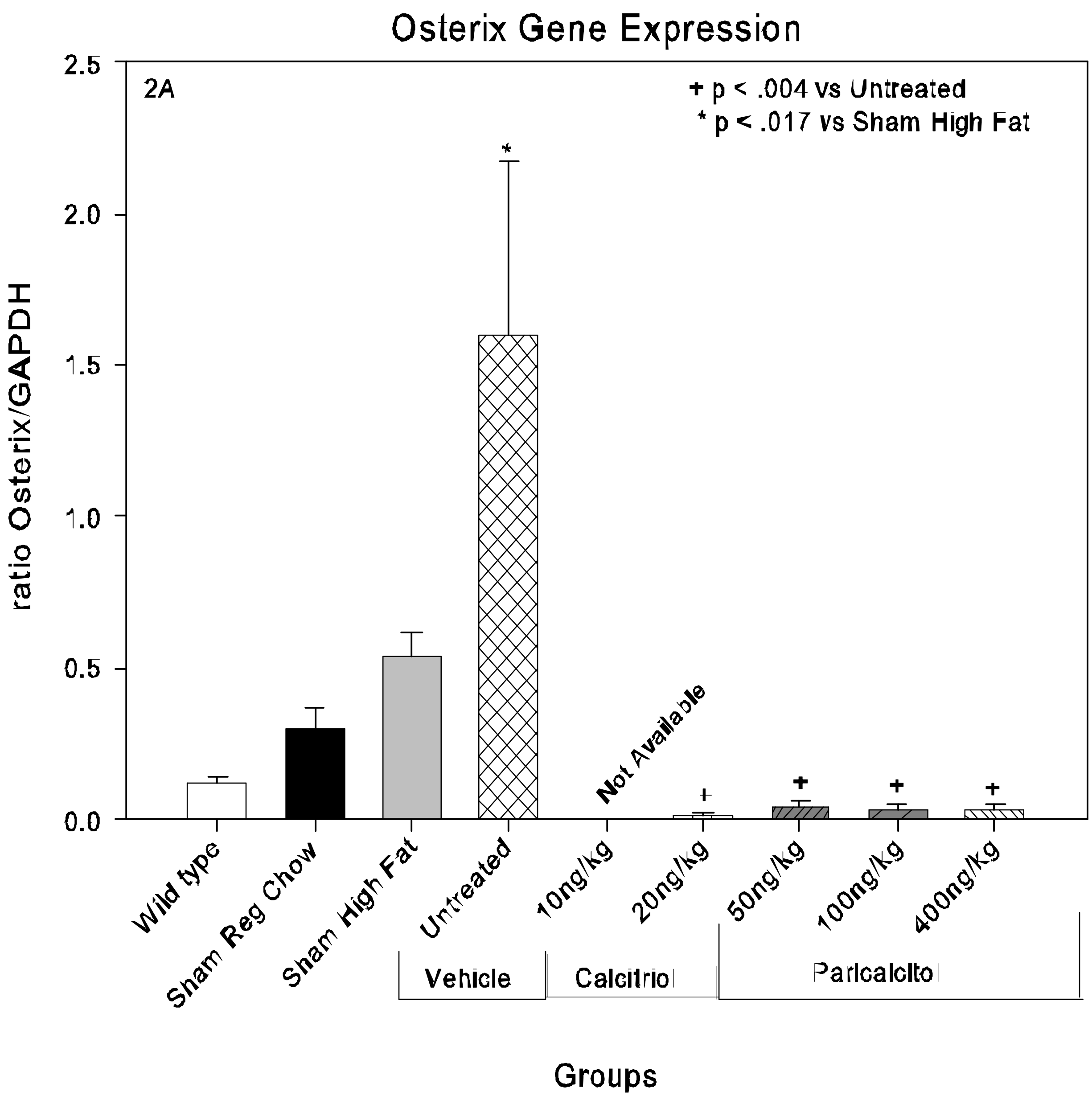
A= 28 wk veh,
B, C = 40, 80, doxercalciferol

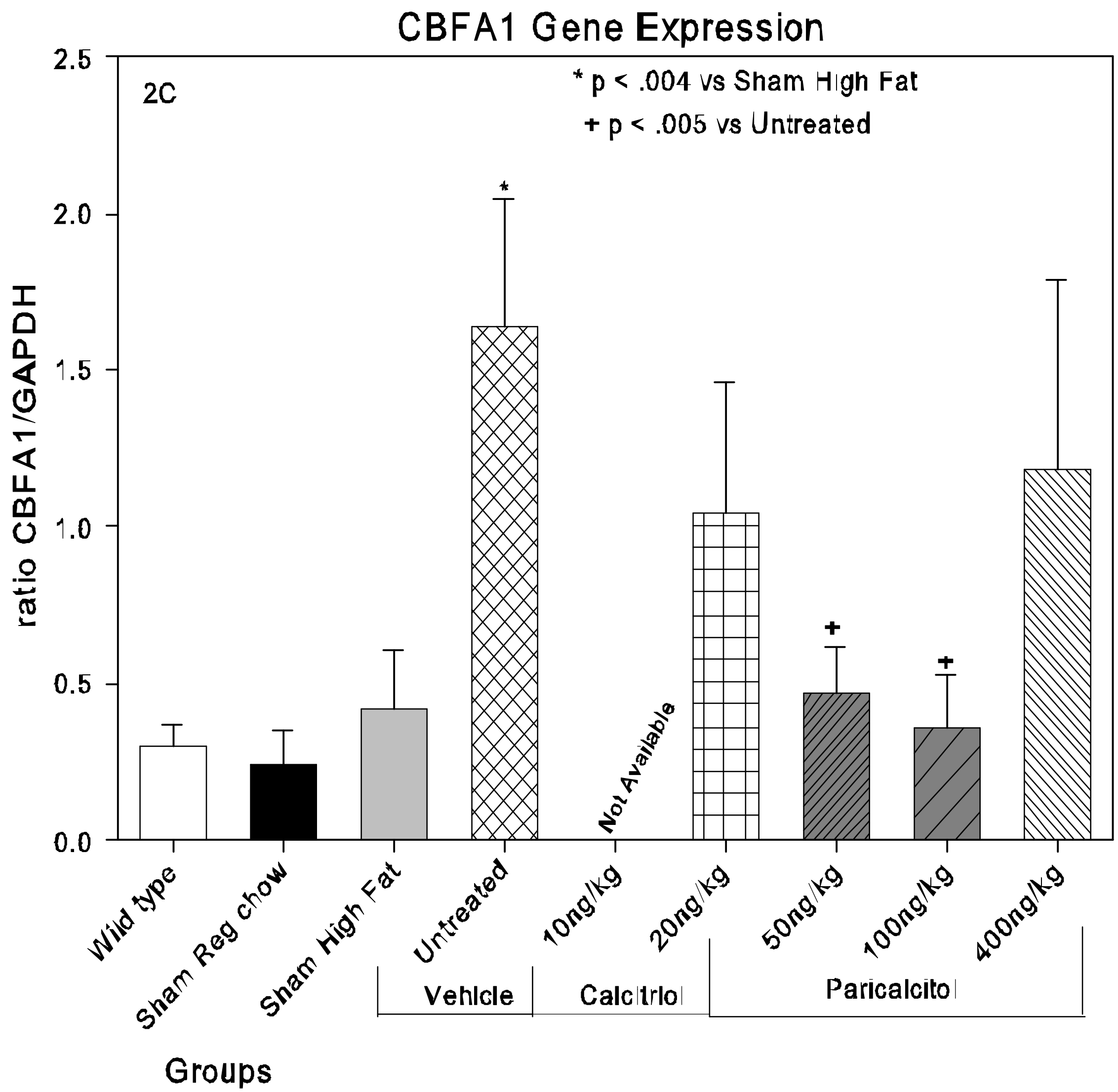
Mathews et al, ASN 2007

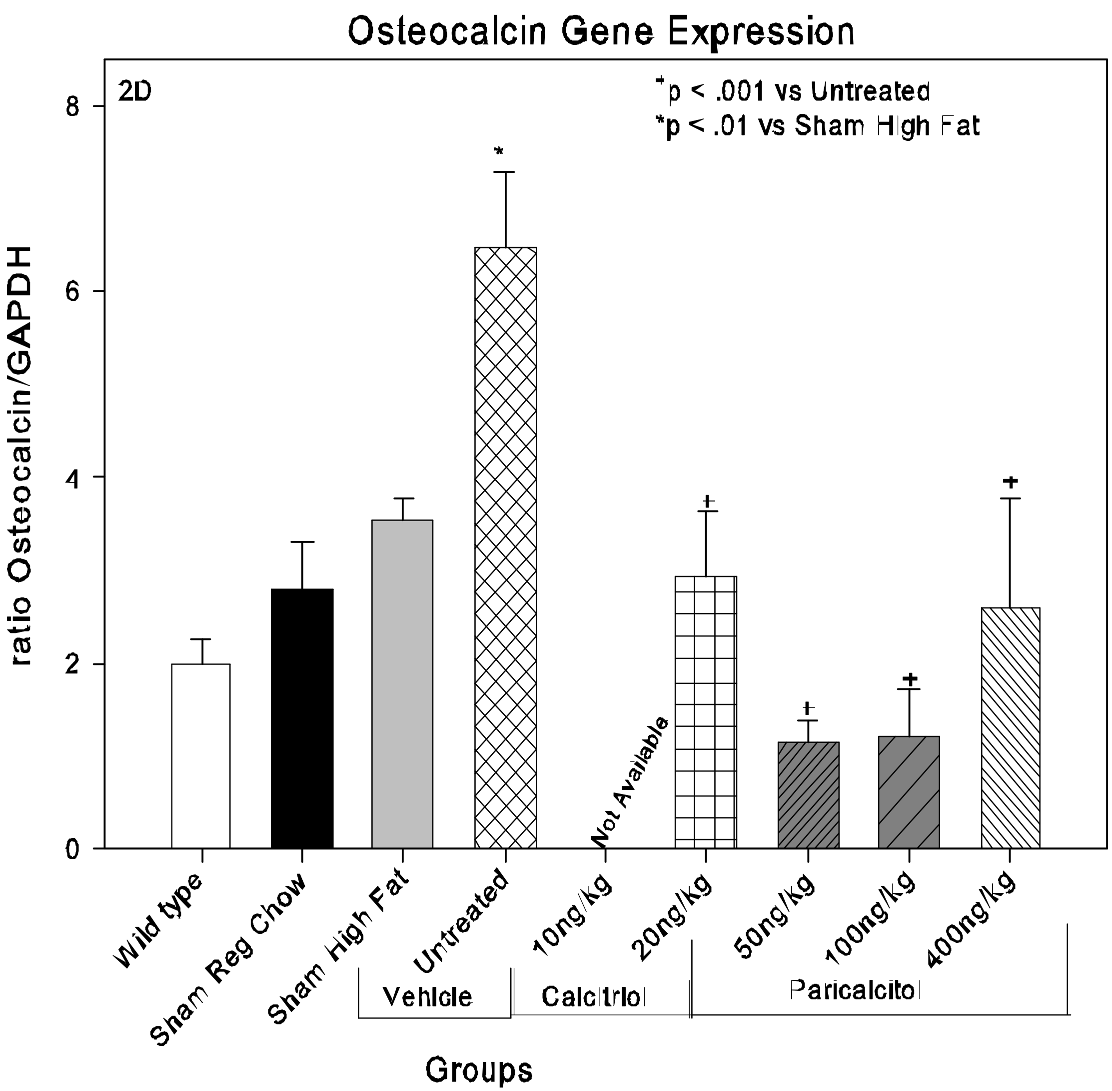
D = 80 doxercalciferol/3% SC
E = 3% SC (sevelamer carbonate)

Mechanisms of VDRA on VC

- **Effect of paricalcitol is a class effect and not due to selective VDR activation**
- **PRIMARY**
  - Inhibition of osteoblastic gene expression in the aorta
- **SECONDARY**
  - Stimulation of bone formation in the adynamic bone disorder of diabetic CKD


Osterix Gene Expression
2A
+ p < .004 vs Untreated
* p < .017 vs Sham High Fat
ratio Osterix/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
*
Not Available
+
+
+
+
Wild type
Sham Reg Chow
Sham High Fat
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paricalcitol
Groups


CBFA1 Gene Expression
2C
* p < .004 vs Sham High Fat
+ p < .005 vs Untreated
ratio CBFA1/GAPDH
0.0
0.5
1.0
1.5
2.0
2.5
Not Available
Wild type
Sham Reg chow
Sham High Fat
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paricalcitol
Groups



Osteocalcin Gene Expression
2D
+p < .001 vs Untreated
*p < .01 vs Sham High Fat
ratio Osteocalcin/GAPDH
0
2
4
6
8
*
+
+
+
+
Not Available
Wild type
Sham Reg Chow
Sham High Fat
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paricalcitol
Groups

Mechanisms of VDRA on VC

- Cultures of human aortic vascular smooth muscle cells derived from atherosclerotic donors demonstrate osteoblastic transition and PO4 stimulation of matrix mineralization due to induction of osterix. Physiologic levels of doxercalciferol and paricalcitol inhibit PO4 induced mineralization due to increased myocardin and decreased osteoblast transition.

Mechanisms of VDRA on VC

- **Effect of paricalcitol is a class effect and not due to selective VDR activation**
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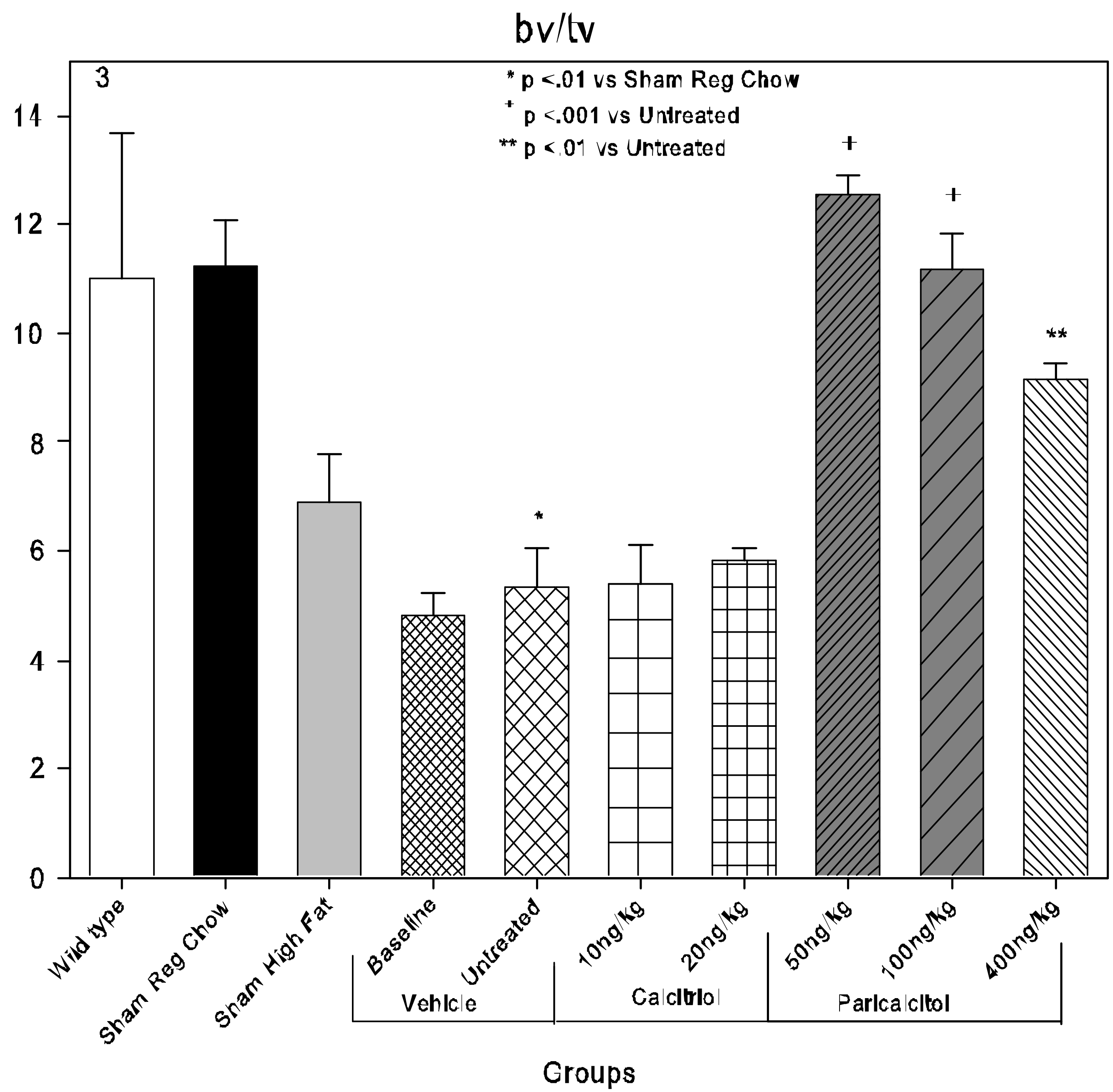
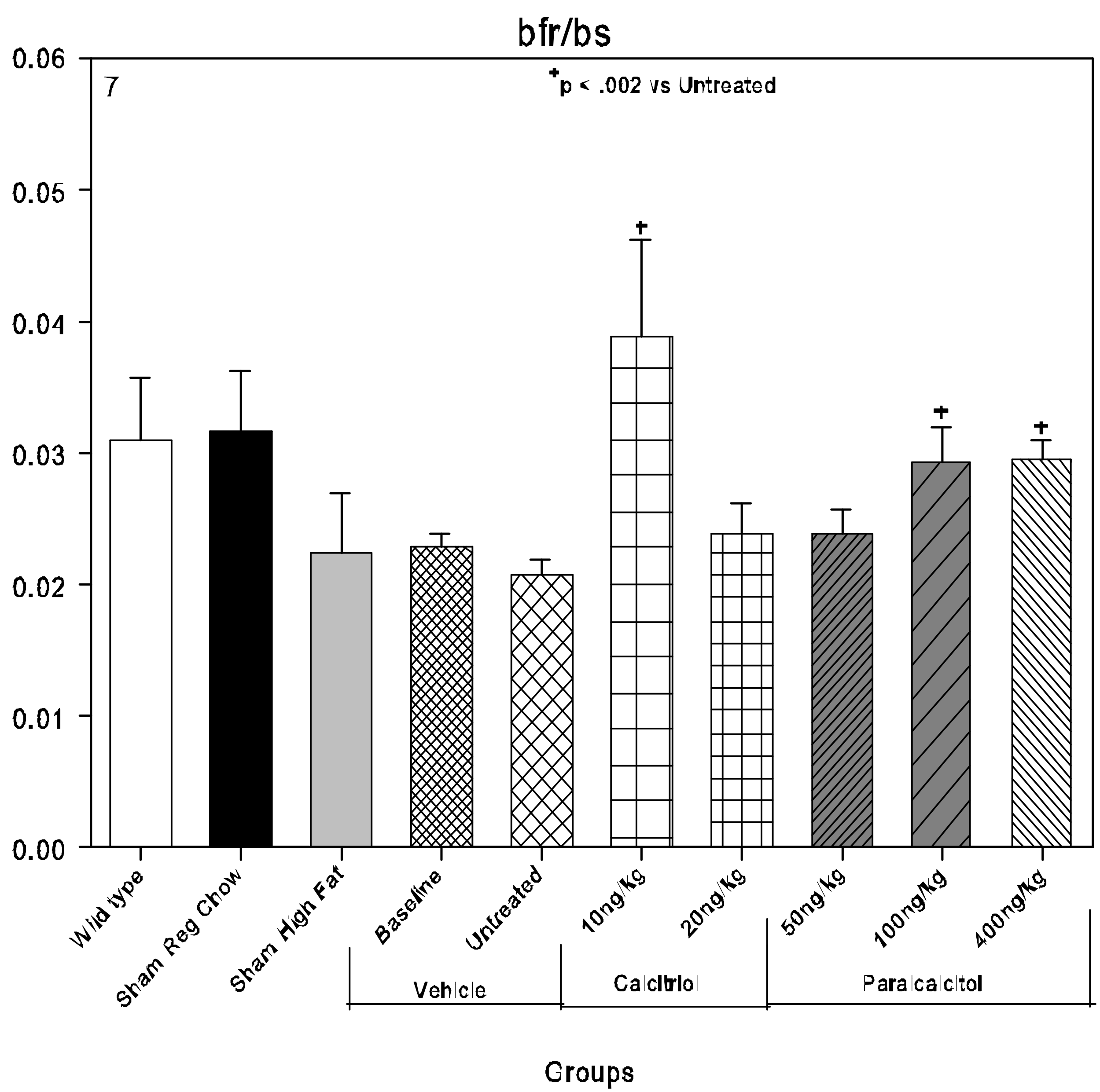
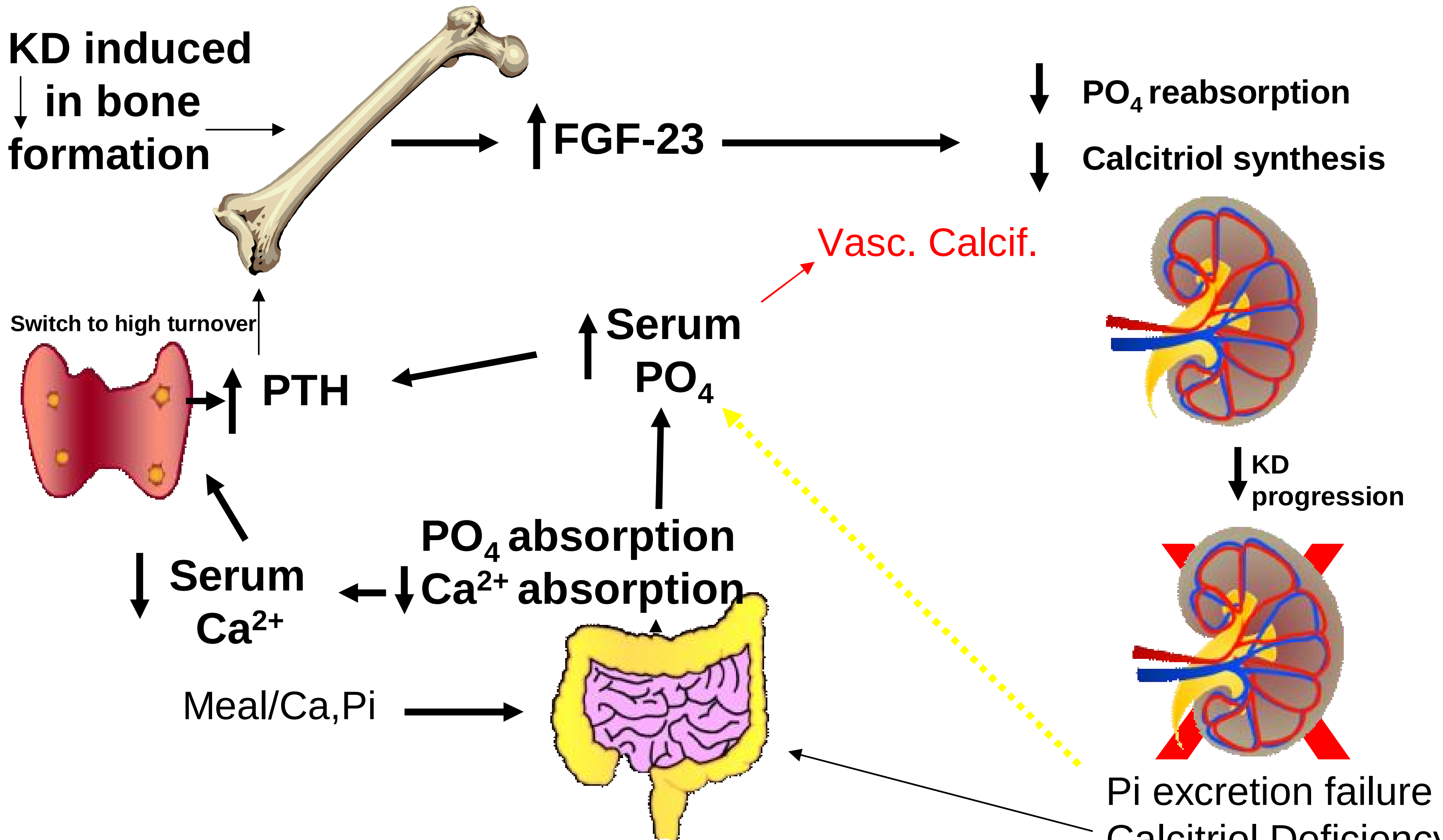
Skeletal Histomorphometry

- Bone remodeling from JASN paper


bv/tv
3
* p <.01 vs Sham Reg Chow
+ p <.001 vs Untreated
** p <.01 vs Untreated
0
2
4
6
8
10
12
14
Wild type
Sham Reg Chow
Sham High Fat
Baseline
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paricalcitol
Groups



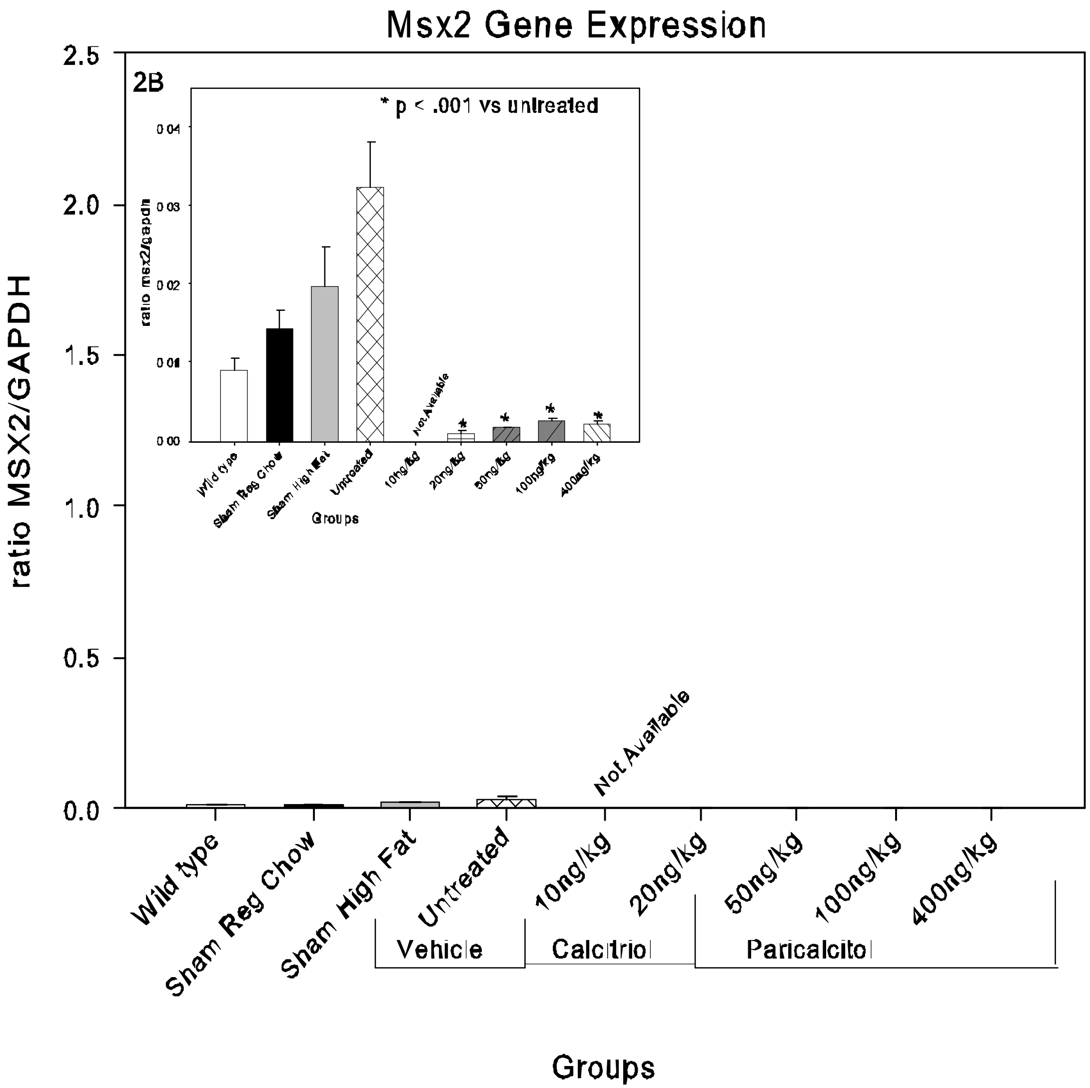
bfr/bs
7
+p < .002 vs Untreated
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Wild type
Sham Reg Chow
Sham High Fat
Baseline
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paralcalcitol
Groups



KD induced
↓ in bone
formation
↑FGF-23
↓ PO_4 reabsorption
↓ Calcitriol synthesis
Vasc. Calcif.
Switch to high turnover
↑ PTH
↑ Serum
PO_4
KD
progression
PO_4 absorption
↓ Ca^{2+} absorption
↓ Serum
Ca^{2+}
Meal/Ca,Pi
Pi excretion failure
Calcitriol Deficiency

Summary

- VDRAs at doses relevant to their clinical indication are protective against atherosclerotic vascular calcification in CKD
- Their primary mechanism of action appears to be inhibition of osteoblastic gene expression in atherosclerotic aortae
- This is opposite to the actions of CKD and hyperphosphatemia
- Secondary mechanisms related to effects on skeletal remodeling
- Differential actions on vascular versus “true osteoblastic cells
- Physiologic actions of VDRA are to stimulate the VSMC cell contractile phenotype repressing atherosclerotic stimulated transition phenotypes


Msx2 Gene Expression
ratio MSX2/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
2B
* p < .001 vs untreated
ratio msx2/gapdh
0.04
0.03
0.02
0.01
0.00
Not Available
Wild type
Sham Reg Chow
Sham High Fat
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Groups
Not Available
Wild type
Sham Reg Chow
Sham High Fat
Untreated
10ng/kg
20ng/kg
50ng/kg
100ng/kg
400ng/kg
Vehicle
Calcitriol
Paricalcitol
Groups

Outline (committee has narrow needs: biphasic aspect of dose response, no vit D, 25-OH defic., CV mort)

- Anatomy of VC
- Focus on Vit D defic - And VC, nl vasc to vasc calcific, ion conc, (Ca Pi)
- Osteoblast diff of VSMC
- 25-OH levels 30,40,50ng/ml of 25-OH, what levels are toxic

Large Observational Studies

- Two demonstrate survival advantage in ESKD with exposure to VDRA, and one shows an advantage of paricalcitol over native hormone
- One study shows survival advantage with doxercalciferol

Vascular Calcification Data

- Calcitriol and paricalcitol at doses equivalent to those used in the clinic were protective against the development of vascular calcification.
- Doses selected on basis of low dose able to suppress PTH levels.