

Warfarin use in chronic kidney disease: a risk factor for vascular calcification?

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Case

- 76M
- PMHx
 - Diabetes Mellitus
 - Hyperlipidemia
 - Ischemic cardiomyopathy with R>L heart failure
 - Atrial fibrillation (CHADS 3)
 - Stage 4-5 CKD

A Case

■ Meds

- Furosemide
- Atorvastatin
- Bisoprolol
- Valsartan
- Amiodarone
- Allopurinol
- Ferrous sulfate
- Lanthanum carbonate
- Sodium bicarbonate
- Vitamin D
- **Warfarin**

A Case

- Presented for CKD follow-up
- eGFR 12 ml/min
- Patient's wife: "Can I show you something on his legs?"



A case

- Diagnosis: Calciphylaxis
 - AKA calcific uremic arteriopathy
 - syndrome of ischemic skin ulceration and necrosis that occurs in tissues supplied by blood vessels with dense medial calcification
 - Incidence ~1-4% among patients with ESRD

A case

- Risk factors for calciphylaxis
 - Hypercalcemia
 - Hyperphosphatemia
 - Hyperparathyroidism
 - Inadequate dialysis
 - Warfarin (**11.4-fold increased risk!**)

What does warfarin have to do with vascular calcification?

Objectives

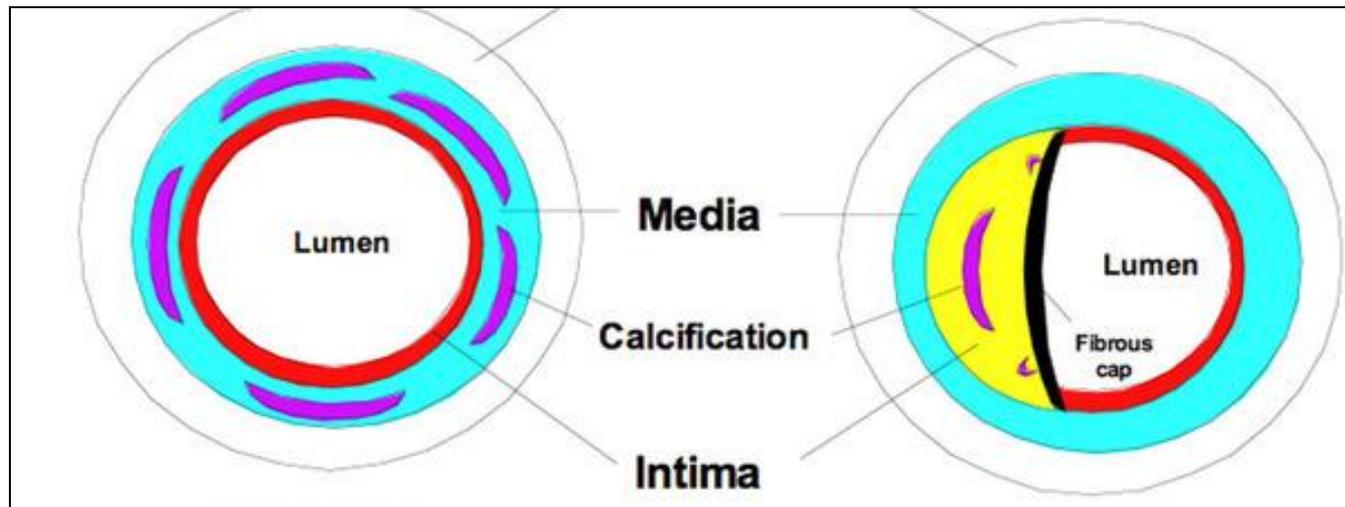
- Discuss the prevalence and mediators of vascular calcification among CKD patients
- Discuss the role of vitamin K deficiency and warfarin use in promoting vascular calcification
- Discuss ongoing studies of the effect of vitamin K2 supplementation on reducing vascular calcification

Vascular calcification

- Intimal vs. medial

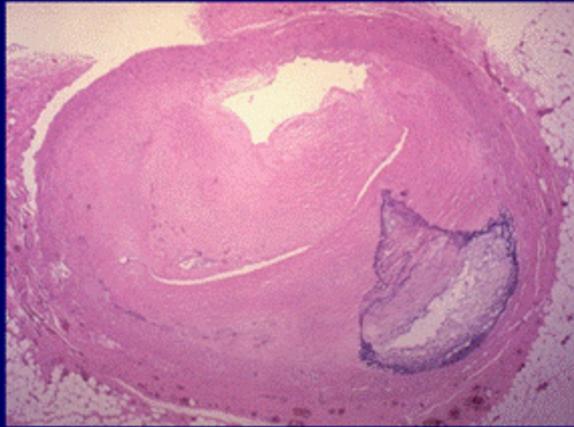
MEDIAL CALCIFICATION

INTIMAL CALCIFICATION



Vascular calcification

Intima and media calcifications : light microscopy aspects



a) intima calcification



b) media calcification

Vascular calcification

- Medial calcification can be seen in non-CKD patients (eg. diabetic), but is most common in patients with CKD and ESRD

What is different about CKD patients?

■ Traditional risk factors

- Hypertension
- Diabetes
- Hyperlipidemia
- Family history
- Age
- Smoking

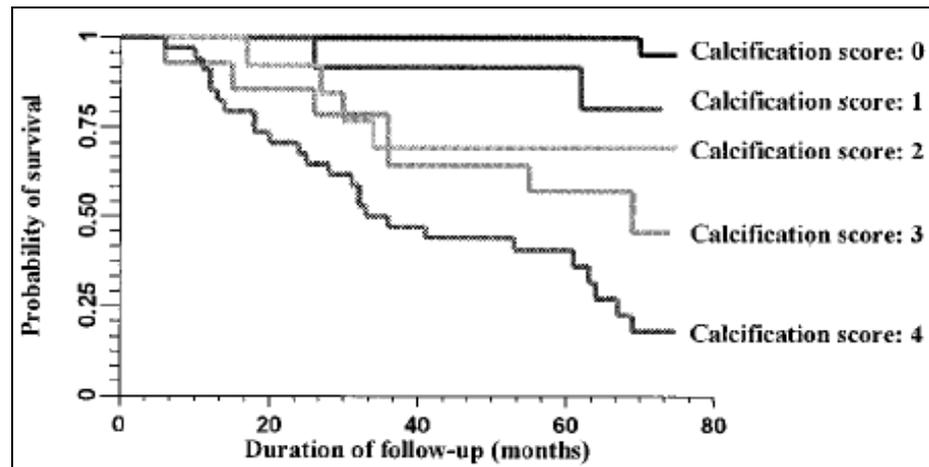
■ Non-traditional risk factors

- Altered mineral metabolism
- Uremia
- Proteinuria
- Anemia
- Inflammation

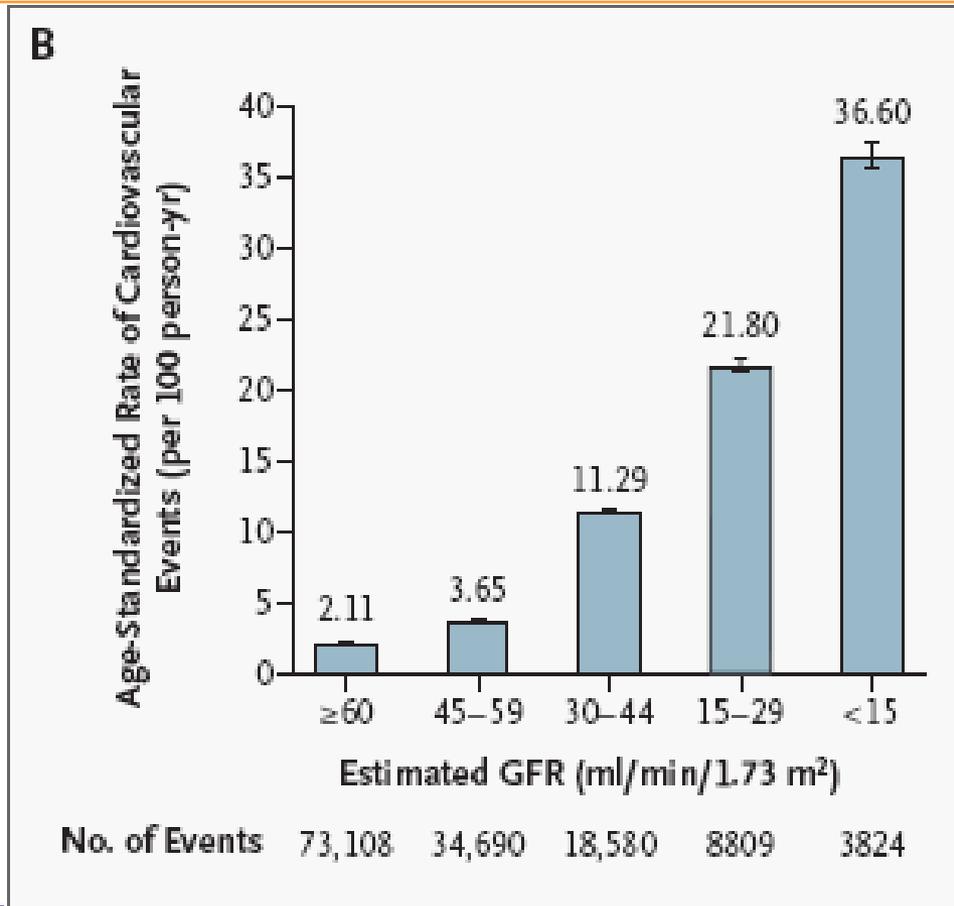


Why is medial calcification so bad?

- Causes stiffening of the vascular wall, reducing arterial compliance
- Contributes to LVH and decreased coronary perfusion, which are associated with increased CV mortality

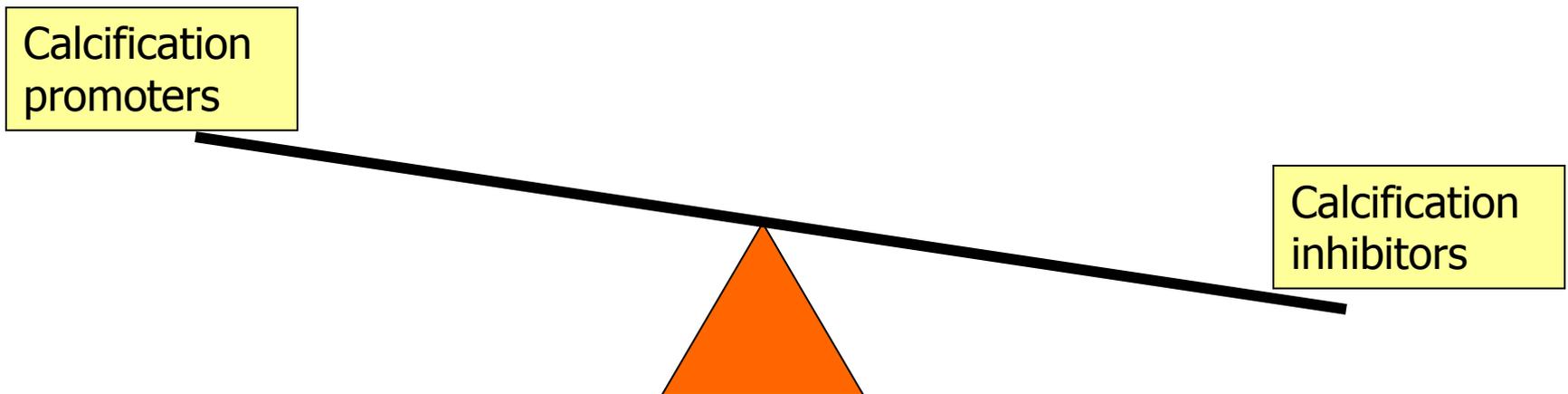


Relationship between eGFR and cardiovascular disease



Why do patients develop vascular calcification?

- Extent of vascular calcification depends on the balance between factors that favour calcification and those that inhibit it

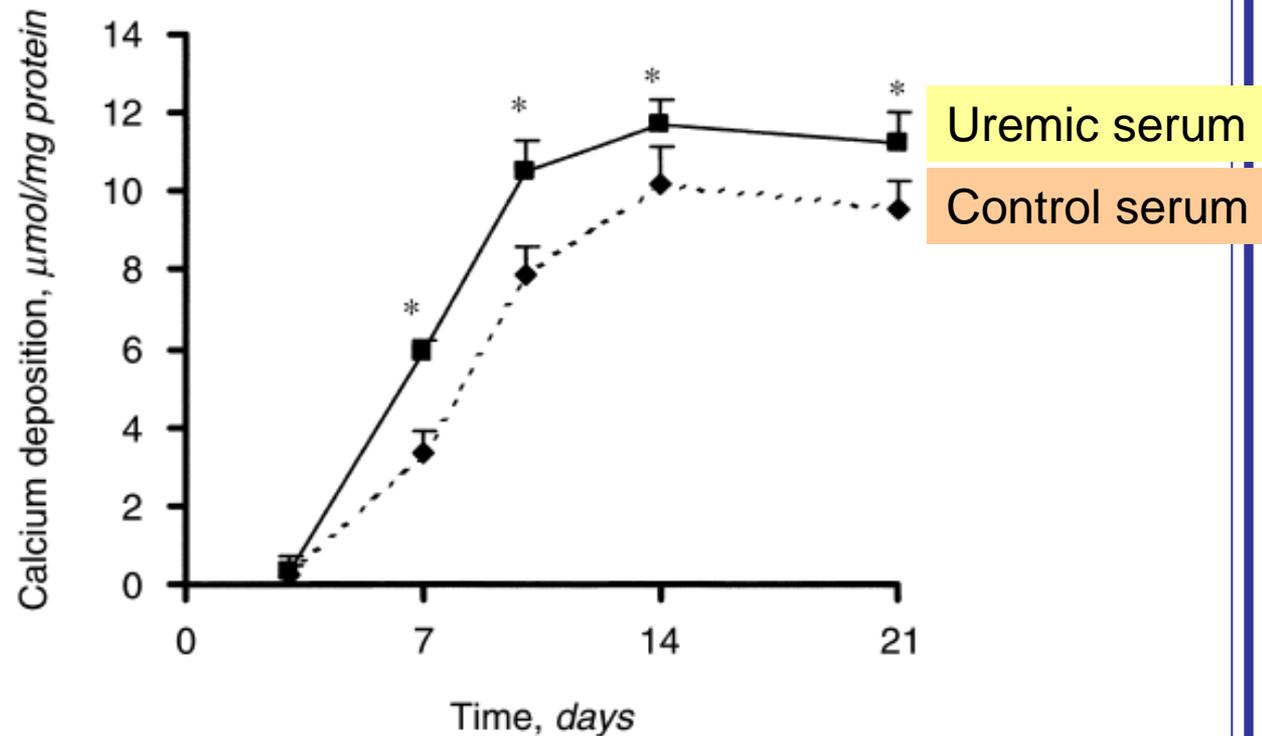


Promoters of vascular calcification

- Uremia
- Hyperphosphatemia
- Hypercalcemia/calcium load
- PTH
- Age
- Diabetes
- Inflammation

Uremic serum promotes vascular calcification

- Incubated bovine vascular smooth muscle cells with calcification media
- Cultured in the presence of control serum or uremic serum
- Measured calcium deposition
- Measured osteopontin expression



Phosphate promotes vascular calcification

- Incubated bovine vascular smooth muscle cells with increasing concentrations of phosphate
- Measured osteopontin expression

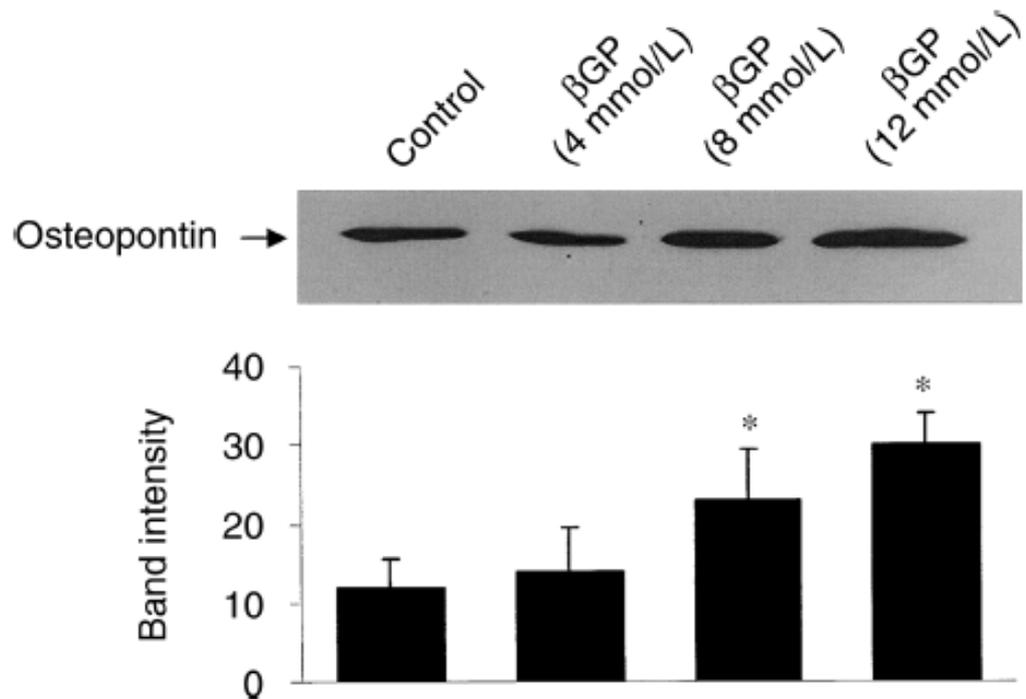
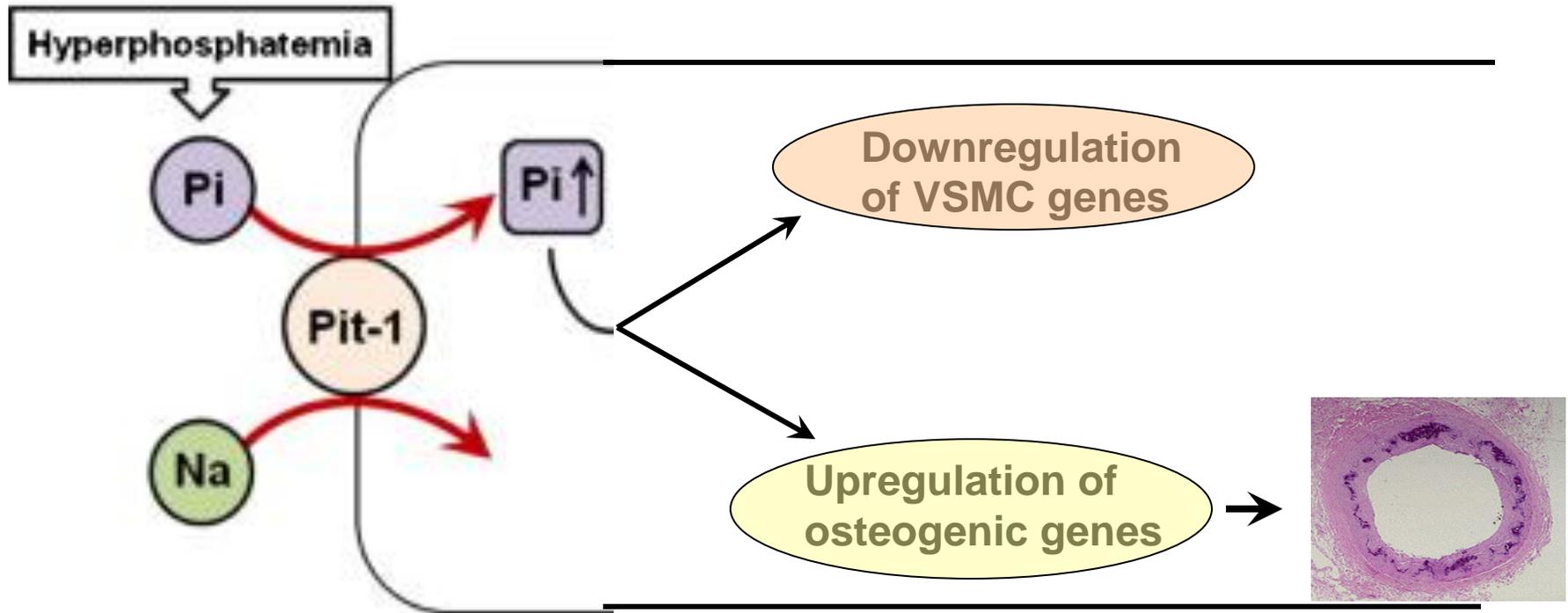
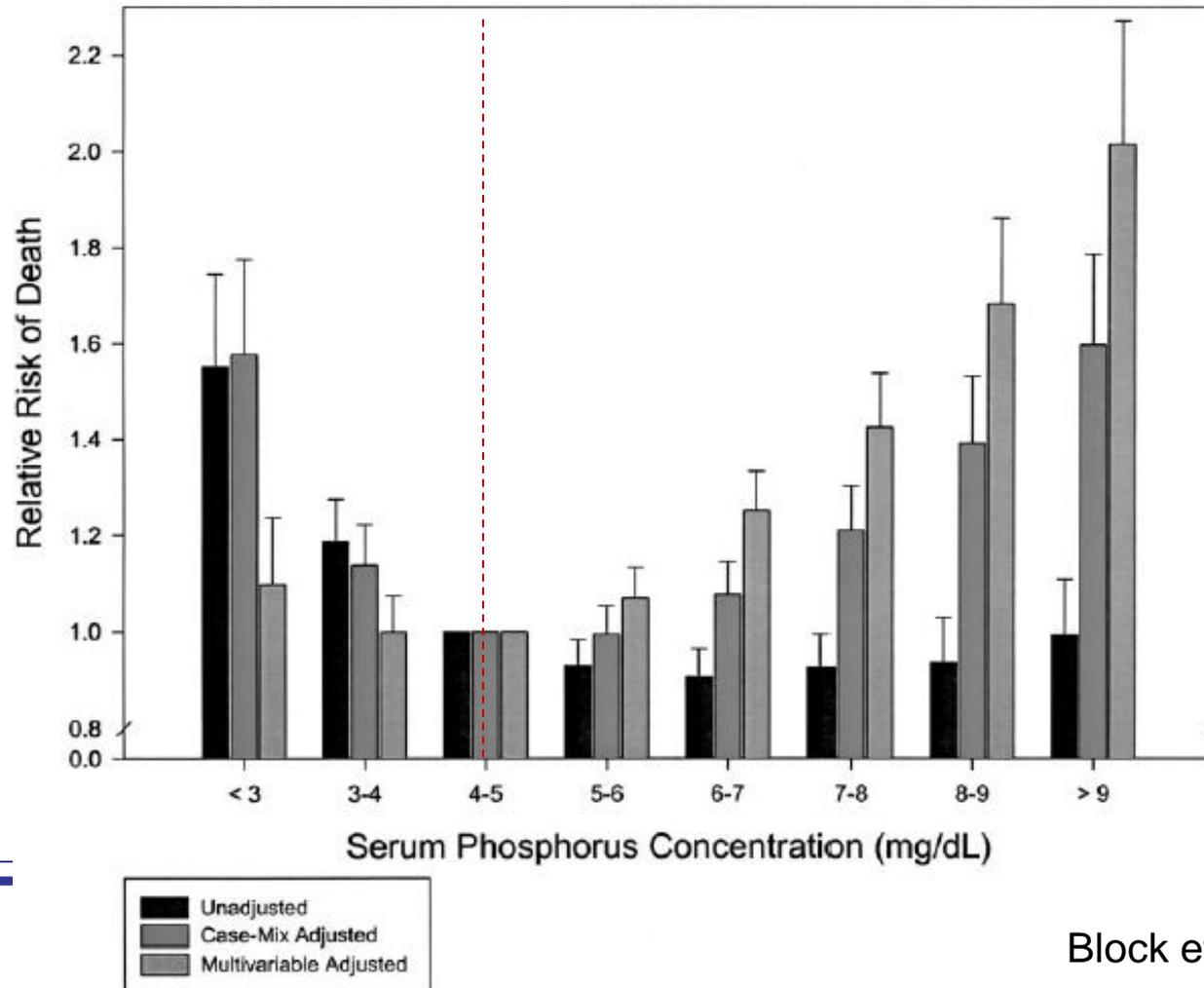


Fig. 1. Western blot analysis of β -glycerophosphate (β GP)-induced osteopontin expression in bovine vascular smooth muscle cells (BVSMC).

Phosphate promotes vascular calcification



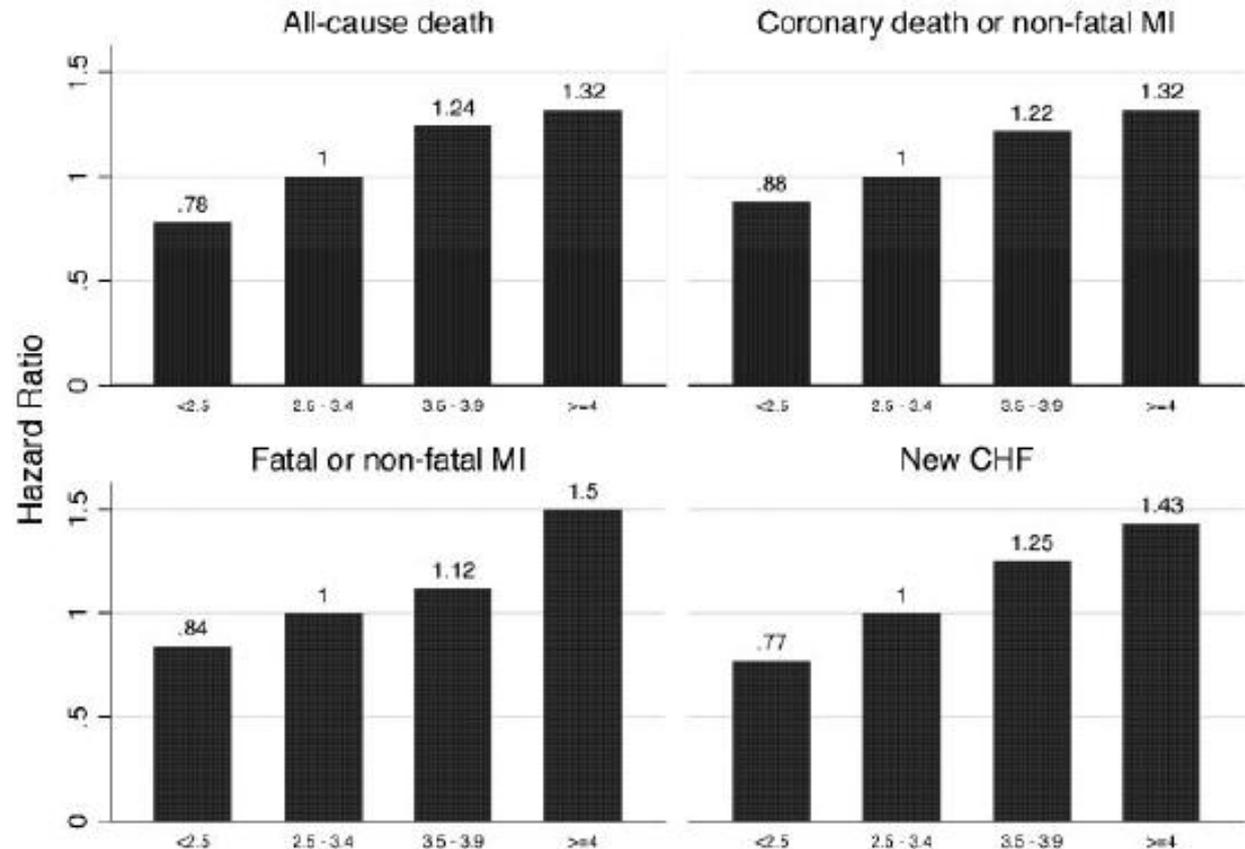
Do phosphate levels matter in the CKD/ESRD population?



Do phosphate levels matter in the non-CKD population?

CARE trial

- Post-hoc analysis
- Divided into 4 groups by serum phosphate (mmol/L):
 - <0.83
 - 0.83-1.1
 - 1.1-1.3
 - >1.3



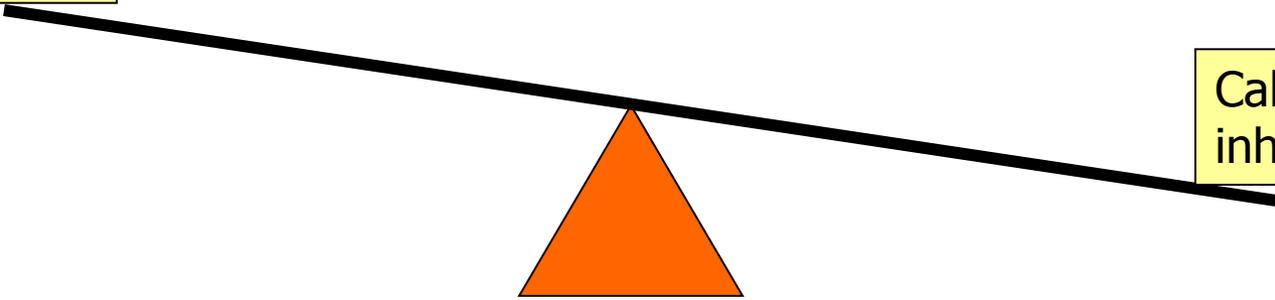
Mediators of vascular calcification

- Uremia
- Phosphate
- Others

Calcification
promoters

??

Calcification
inhibitors



Inhibitors of calcification

- Matrix Gla protein
- Pyrophosphate
- Fetuin-A
- Osteoprotegerin

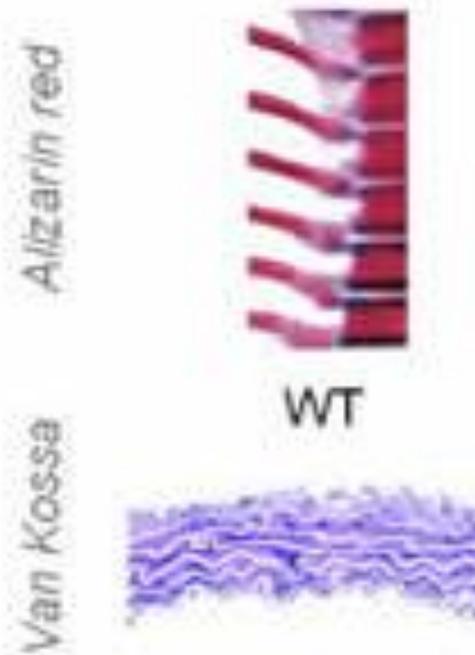
Inhibitors of vascular calcification

■ Matrix Gla protein (MGP)

- The major local calcification inhibitor in the arterial media
- Inhibits calcium crystal formation
- A vitamin K-dependent protein
- Requires γ -carboxylation to its active form

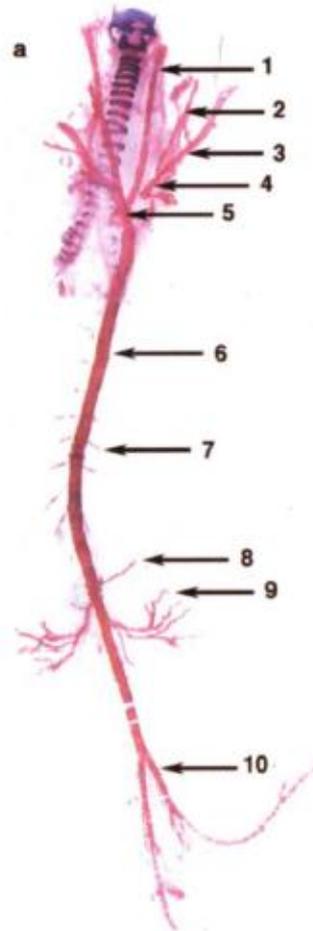
If MGP inhibits medial calcification, does dietary vitamin K deficiency or vitamin K antagonism (warfarin) induce vascular calcification?

How important is MGP?



How important is MGP?

- MGP $-/-$ mice
 - Develop severe medial calcification
 - Die of aortic rupture



How important is MGP?

■ Keutel Syndrome

- Autosomal recessive
- Non-functional MGP
- Patients have pulmonary stenosis and abnormal cartilage calcification
- Extensive medial calcification seen at autopsy



Mediators of vascular calcification

- Uremia
- Hyperphosphatemia
- Hypercalcemia/calcium load
- PTH
- Age
- Diabetes
- Inflammation

MGP

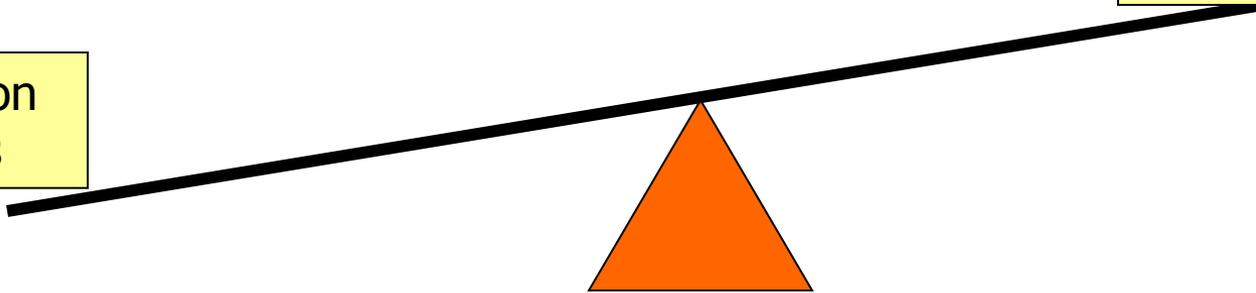
Pyrophosphate

Fetuin-A

Osteoprotegerin

Calcification
promoters

Calcification
inhibitors



Objectives

- Discuss the prevalence and mediators of vascular calcification among CKD patients
- Discuss the role of vitamin K deficiency and warfarin use in promoting vascular calcification
- Discuss ongoing studies of the effect of vitamin K2 supplementation on reducing vascular calcification

Vitamin K subtypes

- Vitamin K1
(phylloquinones)
 - from green leafy vegetables
 - poor oral absorption
 - accumulates predominantly in liver

- Vitamin K2
(menaquinones)
 - from fermented cheeses, soy beans (~10% of intake)
 - excellent absorption
 - accumulates predominantly in extra-hepatic tissue, including arterial vessel wall

Natto!



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Vitamin K

- High doses of vitamin K1 can be converted in extrahepatic tissues to vitamin K2 (MK-4)
 - Warfarin blocks this conversion

Vitamin K

- What are the major vitamin K dependent proteins?
 - Coagulation factors
 - Osteocalcin (involved in bone turnover)
 - Matrix-Gla protein (inhibitor of vascular calcification)

Vitamin K

- Recommended intake = $1\mu\text{g}/\text{kg}/\text{d}$
 - But recommendations are based on hepatic requirements for synthesis of clotting factors
 - At low vitamin K intakes, liver is main site of tissue uptake
 - At high vitamin K intakes, there is increased accumulation in other tissues
 - ie. recommended intake for extrahepatic effects should be higher

Ways to measure vitamin K2 sufficiency

- (1) Measure dietary intake of vitamin K2
- (2) Measure levels of uncarboxylated MGP or osteocalcin (ELISA)

Prevalence of low vitamin K levels

- Pilkey et al, AJKD 2007
 - Measured vitamin K levels in 142 HD patients
 - Results
 - 29% had low K1 levels
 - 93% had significant amounts (at least 20%) of uncarboxylated osteocalcin

Prevalence of low vitamin K levels

- Cranenburg, KI 2012
 - 40 hemodialysis patients
 - Measured vitamin K1 and K2 intake (by food record)
 - Measured vitamin K status
 - Results
 - Intake of K1 and K2 lower than general population
 - High levels of uncarboxylated bone and coagulation proteins in 33/40 patients
 - Very high uncarboxylated MGP levels in all patients

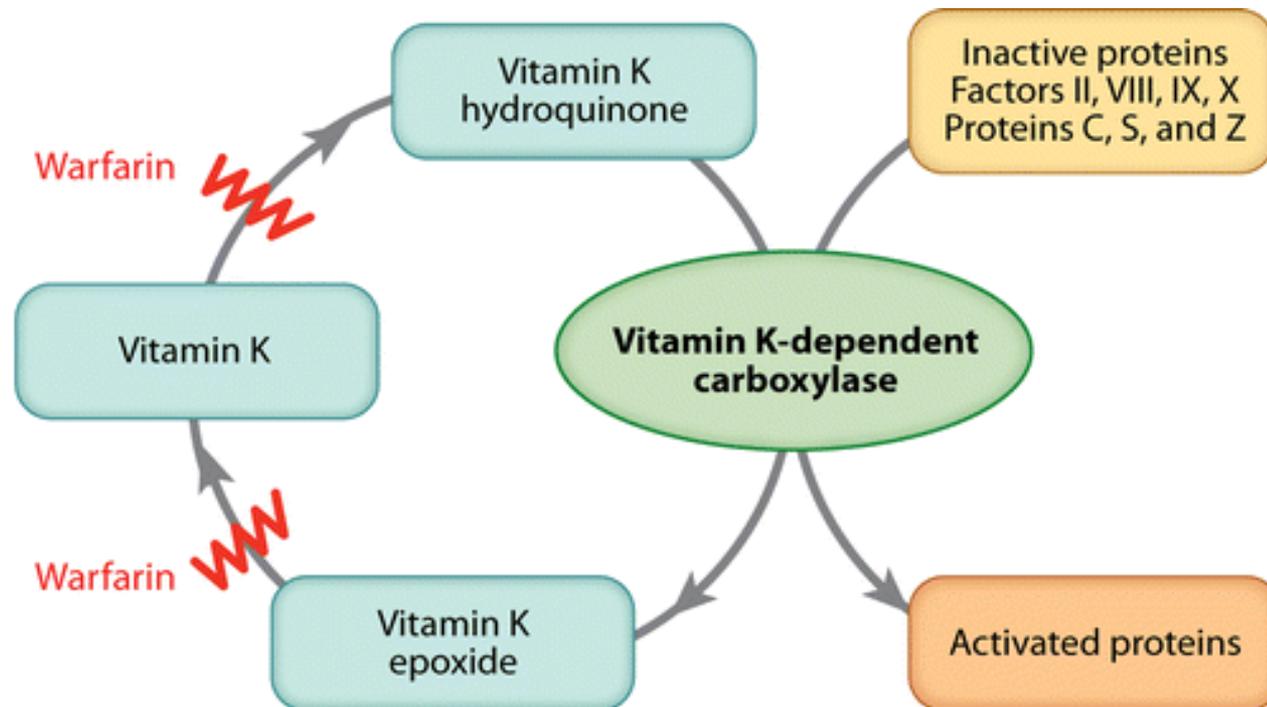
Effect of low Vitamin K intake

- Rotterdam trial
 - 4807 elderly patients observed over time
 - No history of CAD at baseline
 - Divided into tertiles of vitamin K intake
 - Low vitamin K2 intake associated with higher incidence of severe vascular calcification and CV mortality
 - No effect of vitamin K1 intake

Effect of low Vitamin K intake

- Schlieper et al, JASN 2011
 - 188 hemodialysis patients compared with 98 age-matched controls
 - Low levels of carboxylated MGP were associated with
 - higher all-cause mortality (HR 2.2)
 - higher CV mortality (HR 2.7)
 - higher vascular calcification scores

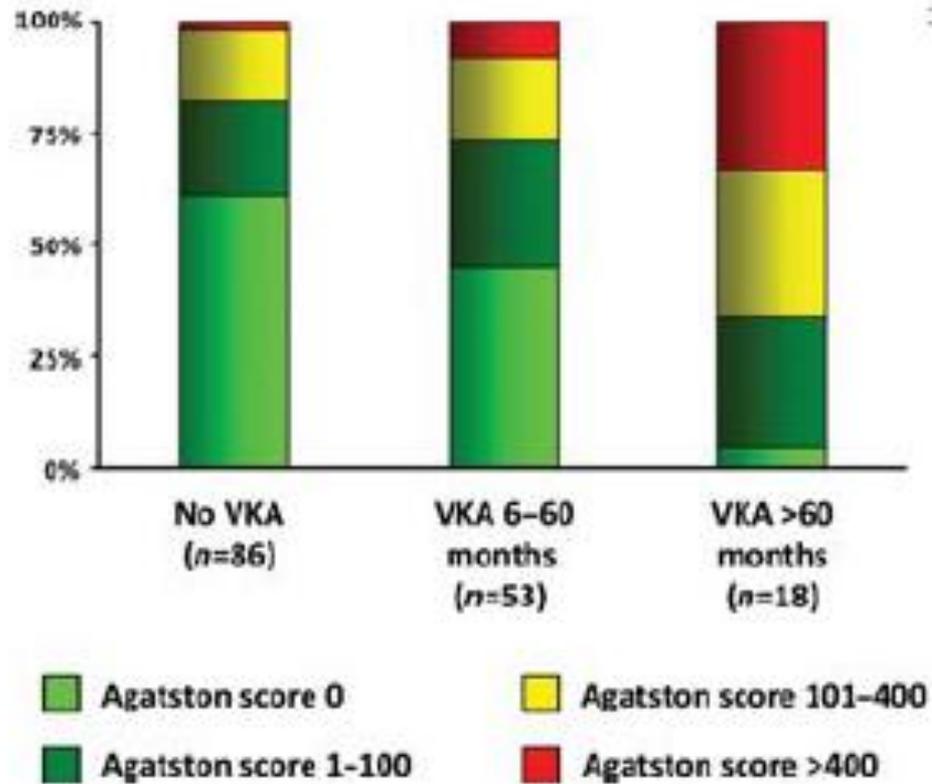
Warfarin: mechanism of action



Effect of Warfarin

- Observational study of 157 patients with A-fib but without significant CV disease
- 71 (45%) on warfarin
- No difference in clinical characteristics between patients taking or not taking warfarin

Effect of Warfarin



Effect of Warfarin

- Observational study of 83 patients with calcific aortic valve disease
- 23/83 on warfarin (mean duration 88 months)
- Compared coronary and aortic calcification scores

Table 3. Valvular and coronary calcium scores assessed by multislice spiral computed tomography stratified by anticoagulation status

Variable	Oral Anticoagulants		p Value
	Yes (n = 23)	No (n = 63)	
Valvular Agatston score	2,409.9 ± 1,758.5	1,070.1 ± 1,084.6	0.002
Coronary Agatston score	1,561.3 ± 1,140.5	738.2 ± 977.5	0.024

Effect of Warfarin

- 108 hemodialysis patients, 18 of whom were on warfarin for >18 months

TABLE III

FACTORS INDEPENDENTLY ASSOCIATED WITH ODDS OF HIGHER CATEGORY OF AV CALCIFICATION (ORDINAL REGRESSION)

Factor	Odds ratio (95% CI)	p Value
>18 months warfarin*	3.77 (0.97-14.7)	0.055
Age (10-year increase)	1.69 (1.12-2.55)	0.012
Dialysis vintage (1-year increase)	1.13 (0.98-1.30)	0.089
Calcium (500-mg increments)	1.41 (1.03-1.91)	0.027
Calcitriol (0.25- μ g increments)	1.73 (0.88-3.41)	0.113

The model accounted for 23.3% of the variance (Cox and Snell r^2).

*Reference group was <18 months of warfarin exposure.

Effect of Warfarin: Caveat



- Most of these studies are observational, so one cannot infer causality
 - Possibility #1: Warfarin causes vascular calcification
 - Possibility #2: Warfarin is associated with vascular calcification because patients on warfarin are sicker

Effect of Warfarin

- Rat model of warfarin-induced low K2
 - Rats given warfarin + vitamin K1 supplementation (to prevent bleeding)
 - ie. adequate levels of K1, inadequate levels of K2
 - Induced rapid calcification of rat arteries and heart valves

Effect of Warfarin

- Rat model of warfarin in CKD vs. normal renal fxn



Effect of Warfarin

- Rat model of warfarin in CKD vs. normal renal function
 - CKD rats showed
 - 3x increased calcium concentration in thoracic aorta
 - 8x increase in abdominal aorta
 - 4x increase in renal artery
 - 20x increase in carotid artery
 - Increased pulse pressure and pulse wave velocity

Summary so far

- MGP is a potent inhibitor of vascular calcification
- MGP is dependent on vitamin K to γ -carboxylate it to its active form
- Vitamin K2 is more important than vitamin K1 for γ -carboxylation of extra-hepatic proteins
- Low vitamin K2 levels and warfarin use are associated with increased vascular calcification

Objectives

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How to supplement Vitamin K2?

OPTION #1



OPTION #2



Vitamin K2 Supplementation

- Westenfield et al, AJKD 2012
 - Randomized non-placebo controlled trial
 - 53 hemodialysis patients vs. with 50 age-matched controls
 - 3 parallel groups
 - Vitamin K2 at 45 ug, 135 ug or 360 ug/d for 6 weeks

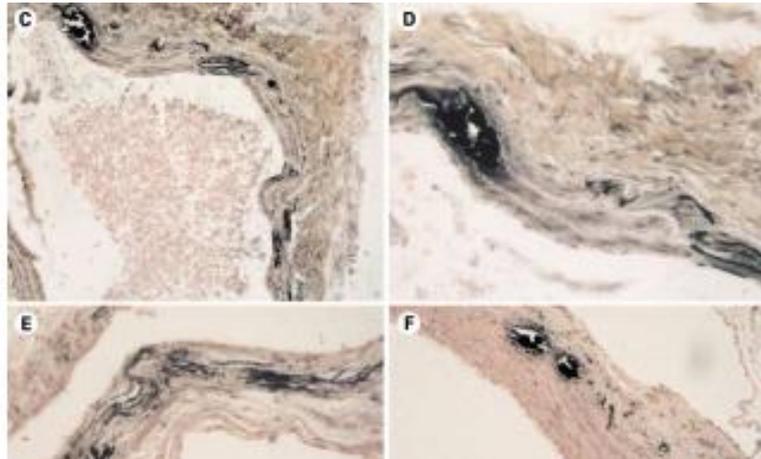
Vitamin K2 Supplementation

- Westenfield et al, AJKD 2012
 - At baseline, hemodialysis patients had
 - 4.5x higher uncarboxylated MGP
 - 8.4x higher uncarboxylated osteocalcin
 - (suggests that most HD patients have a functional vitamin K deficiency)
 - Vitamin K2 induced a dose-dependent and time-dependent decrease in uncarboxylated MGP and osteocalcin

Can Vitamin K2 prevent calcification?

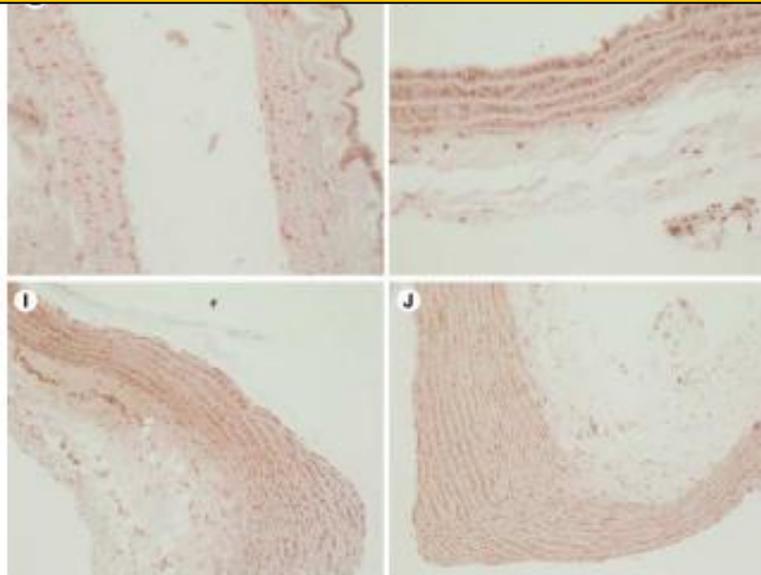
- Rat model comparing
 - Control
 - Warfarin + K1
 - Warfarin + K2

Warfarin +K1

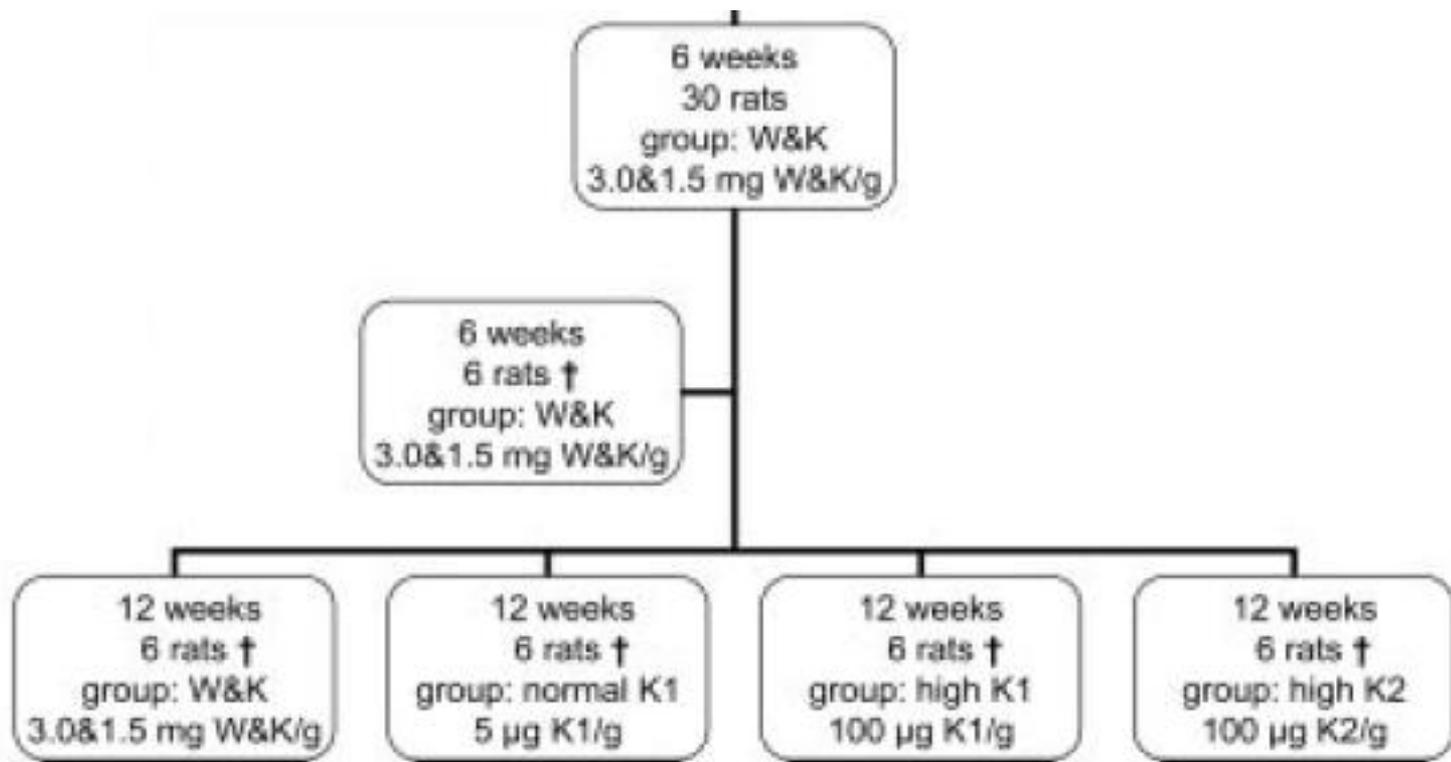


Conclusion: Administering vitamin K2 at the same time as warfarin can prevent vascular calcification

Warfarin +K2



Can Vitamin K2 regress calcification?



Mineralization

uc-MGP

c-MGP

Control



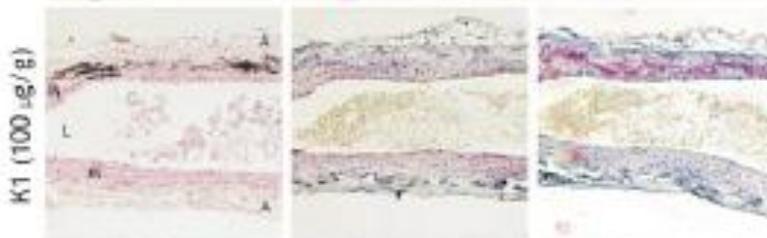
Warfarin



Conclusion: Administering vitamin K2 can regress warfarin-induced vascular calcification



High dose K1



High dose K2



Registered trials on Vitamin K2

Rank	Status	Study
1	Completed	<p>Comparison of Efficacy of Different Dosages Vitamin K2</p> <p>Conditions: Carboxylation Level; Vitamin K-dependent Proteins</p> <p>Interventions: Dietary Supplement: placebo; Dietary Supplement: vitamin K1; Dietary Supplement: vitamin K2</p>
2	Completed	<p>Dose Defining Study for the Administration of Vitamin K2 Supplements in Hemodialysis Patients</p> <p>Condition: Vascular Calcification</p> <p>Intervention: Dietary Supplement: Vitamin K2 supplementation</p>
3	Recruiting	<p>Vitamin K2 Intervention in Patients With Vitamin K Antagonists</p> <p>Condition: Thrombosis</p> <p>Intervention: Dietary Supplement: Vitamin K2</p>
4	Recruiting	<p>The Effects of Vitamin K2 Supplementation on the Progression of Coronary Artery Calcification</p> <p>Condition: Coronary Artery Disease</p> <p>Interventions: Dietary Supplement: Menaquinone-7 (Vitamin K2); Other: Placebo capsules</p>
5	Unknown [†]	<p>Vitamin K2 and Vessel Calcification in Chronic Kidney Disease Patients</p> <p>Conditions: Kidney Diseases; Coronary Artery Calcification</p> <p>Interventions: Drug: Vitamin K2+10µg cholecalciferol; Drug: Vitamin D</p>
6	Completed	<p>Comparison of Absorption of Vitamin K2</p>

Ongoing Studies (1)

- “Vitamin K2 and vessel calcification in CKD patients”
 - Randomized double-blind study
 - N=60 patients with CKD (GFR 15-60 ml/min) with calcium score >10 (Agatston scoring system)
 - Randomized to **vitamin K2** + Vit D3, or Vit D3 alone
 - Primary endpoint – the degree of vessel calcification by imaging at 9 months

Ongoing Studies (2)

- “The effects of vitamin K2 supplementation on the progression of coronary calcification”
 - Randomized, double-blind, placebo-controlled trial
 - N=180 patients with established coronary artery calcification
 - Objective: to investigate whether daily supplementation of Vitamin K2 will lead to a decreased progression-rate of CAC after 24 months of follow-up as compared with placebo

Ongoing Studies (3)

- “Vitamin K2 intervention in patients with Vitamin K antagonists”
 - Randomized placebo-controlled trial
 - N=52 patients on coumadin
 - To receive Vitamin K2 (75 ug/d) or placebo for 4 months
 - Primary objective: to determine whether Vitamin K2 supplementation upsets the balance of anticoagulant treatment
 - Secondary objective: to evaluate if regular consumption of vitamin K2 improves the markers of bone mineralization

“Effect of low-dose vitamin K2 on the stability of oral anticoagulant treatment”

- 18 healthy volunteers given warfarin x 4 weeks
 - Found to have increased levels of UC-MGP
- Then warfarin continued and given increasing doses of MK-7 x 6 weeks
 - Highest dose decreased INR by 40%
 - Lower doses caused clinically relevant lowering of INR in 40-60% of patients

Back to the case...



- Started on daily dialysis to optimize uremic clearance and phosphate removal
- Warfarin stopped
- Started on Vitamin K2
- Developed infection of wound, which persisted despite broad spectrum antibiotics
- Died 1 month later

Summary

- MGP is the major inhibitor of calcification in the blood vessel wall
- MGP is activated (carboxylated) by vitamin K
- MGP may be inactive in the presence of vitamin K2 deficiency or warfarin
- There may be a role for vitamin K2 in the prevention of vascular calcification