Role of vitamin K2 in bone and vascular calcification

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Once upon a time…

a vitamin, once regarded as the Cinderella of fat-soluble vitamins emerged from a single-function "haemostasis vitamin" to a "multi-function vitamin" and arguably the most fascinating of all…

…It's name is vitamin K…

Shearer et al. DeGruyter 2011

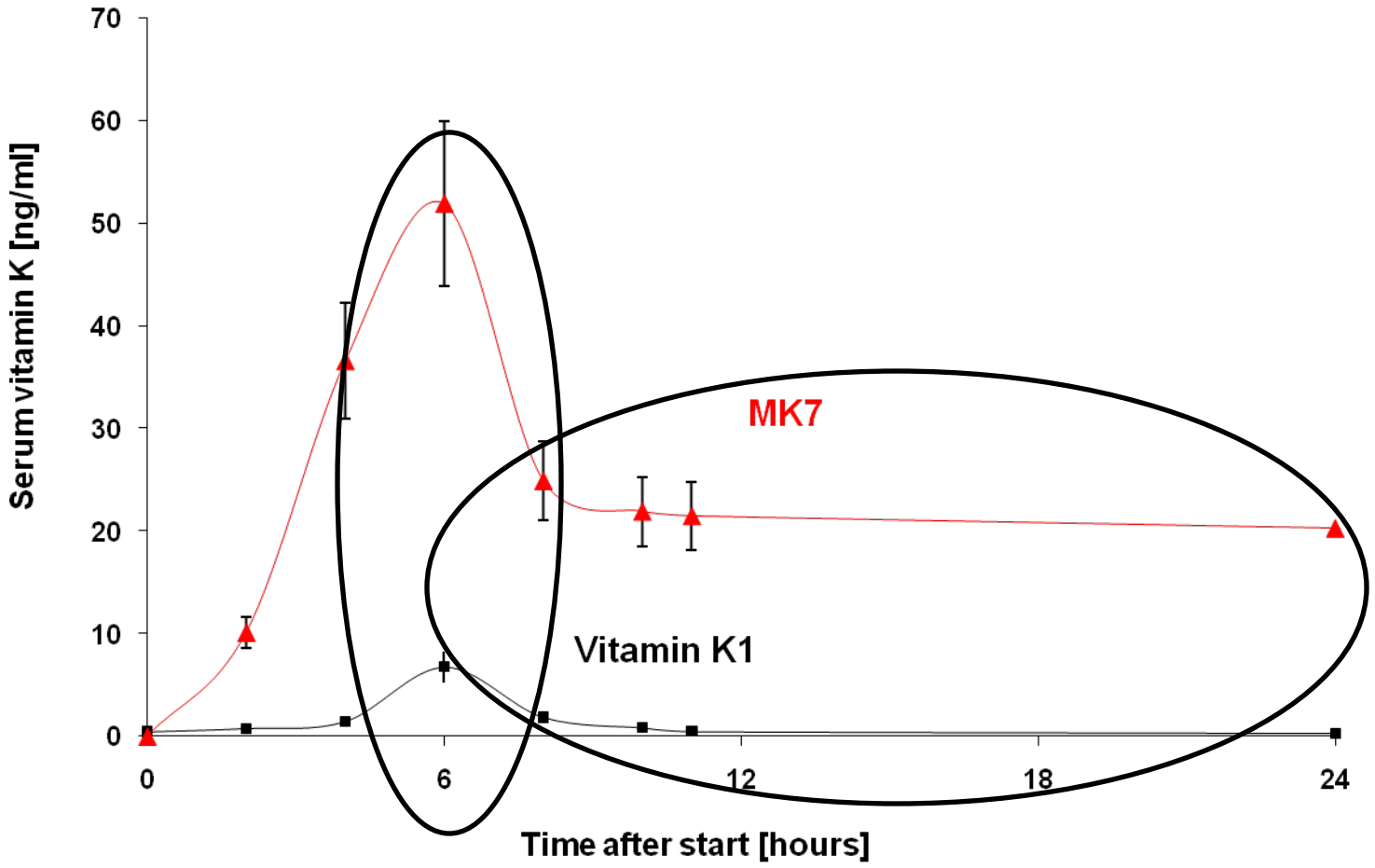
Vitamin K

- Discovered in 1929
- K stands for “koagulation”
- 14 VKD-proteins known
- Activates vitamin K-dependent proteins
- Vitamin K1 and vitamin K2

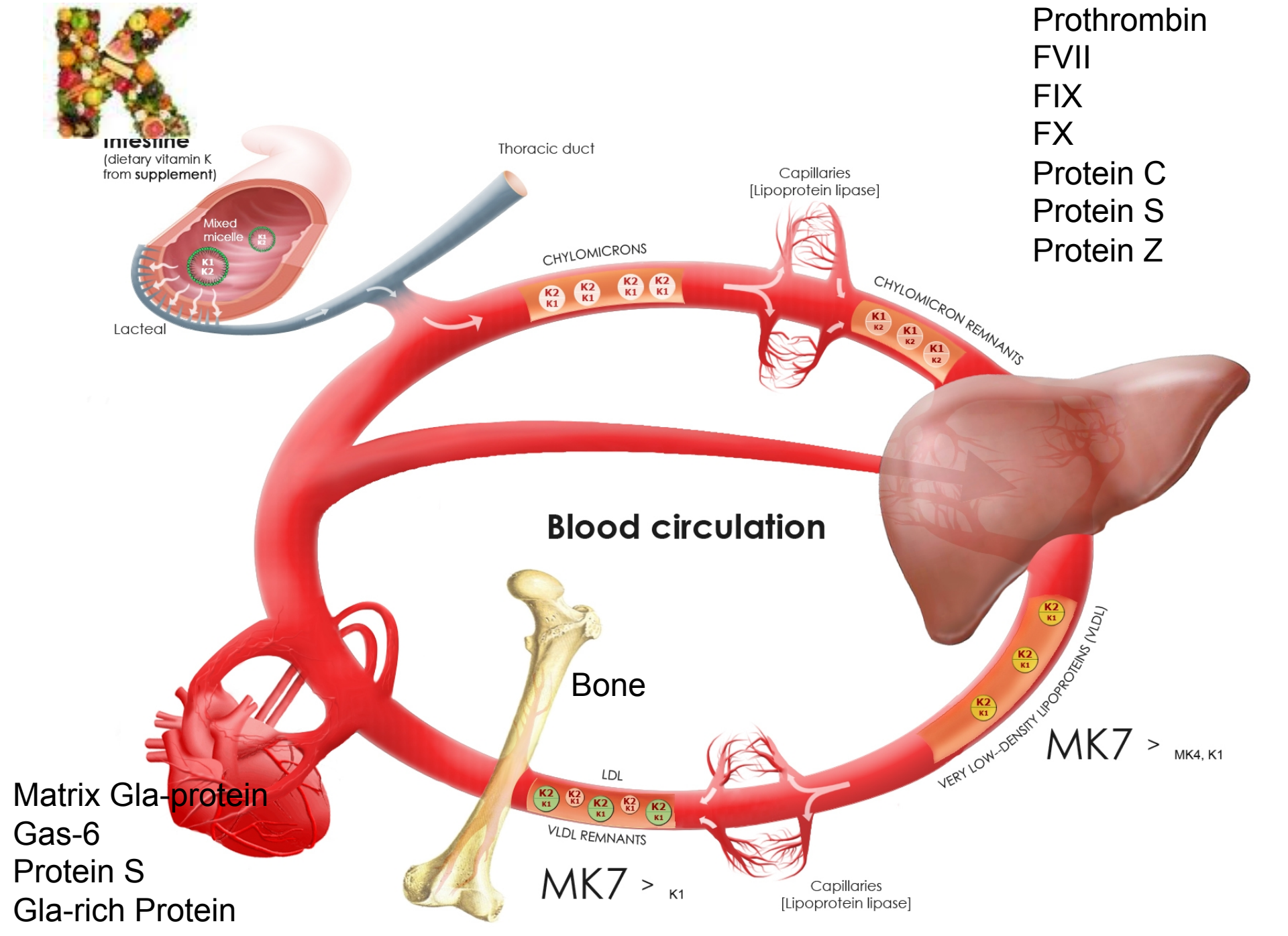
Dam and Doisy shared the **1943 Nobel Prize** for their work on Vitamin K

Henrik Dam **Edward Adelbert Doisy**

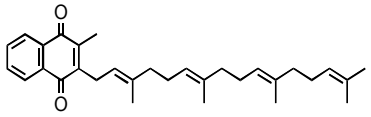
Absorption of natural vitamin K1 from spinach and natural vitamin K2 (MK7) from natto

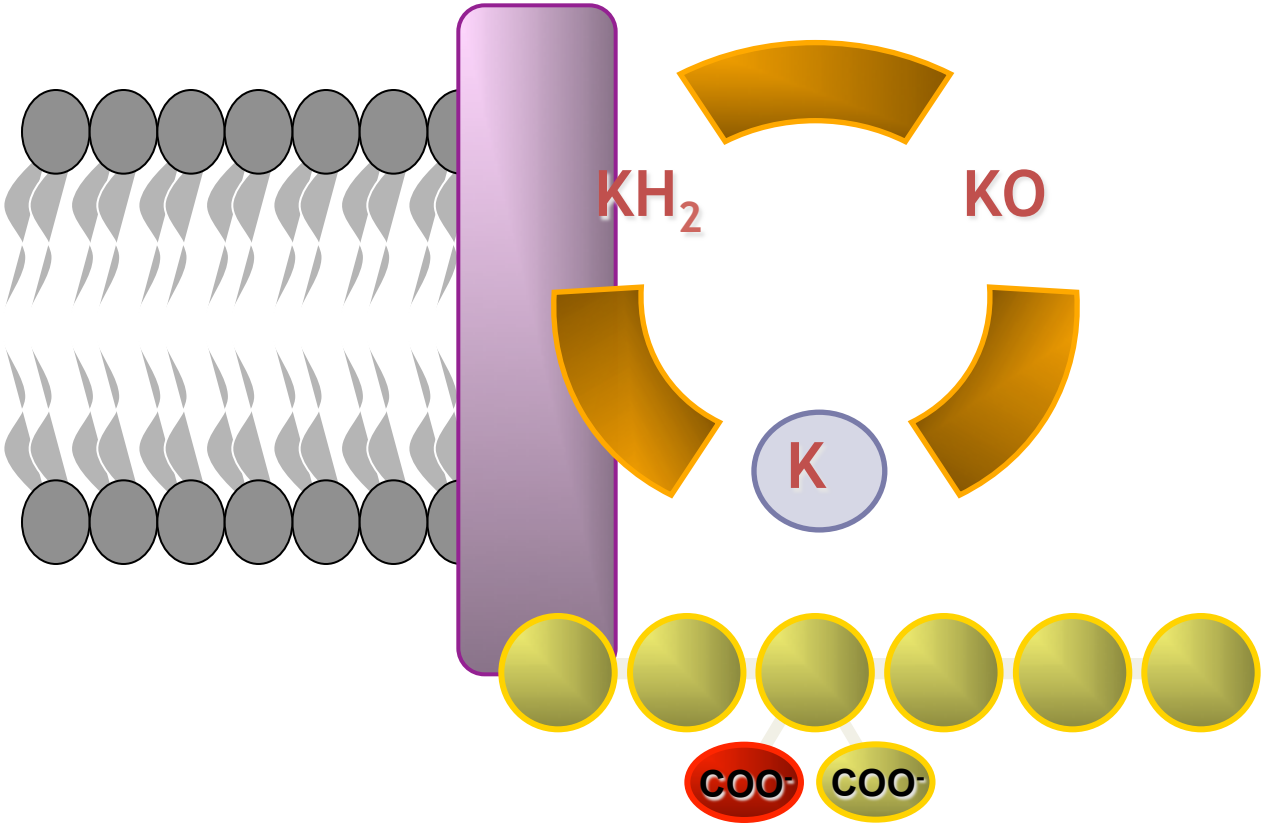


Schurgers et al. Haemostasis 2000

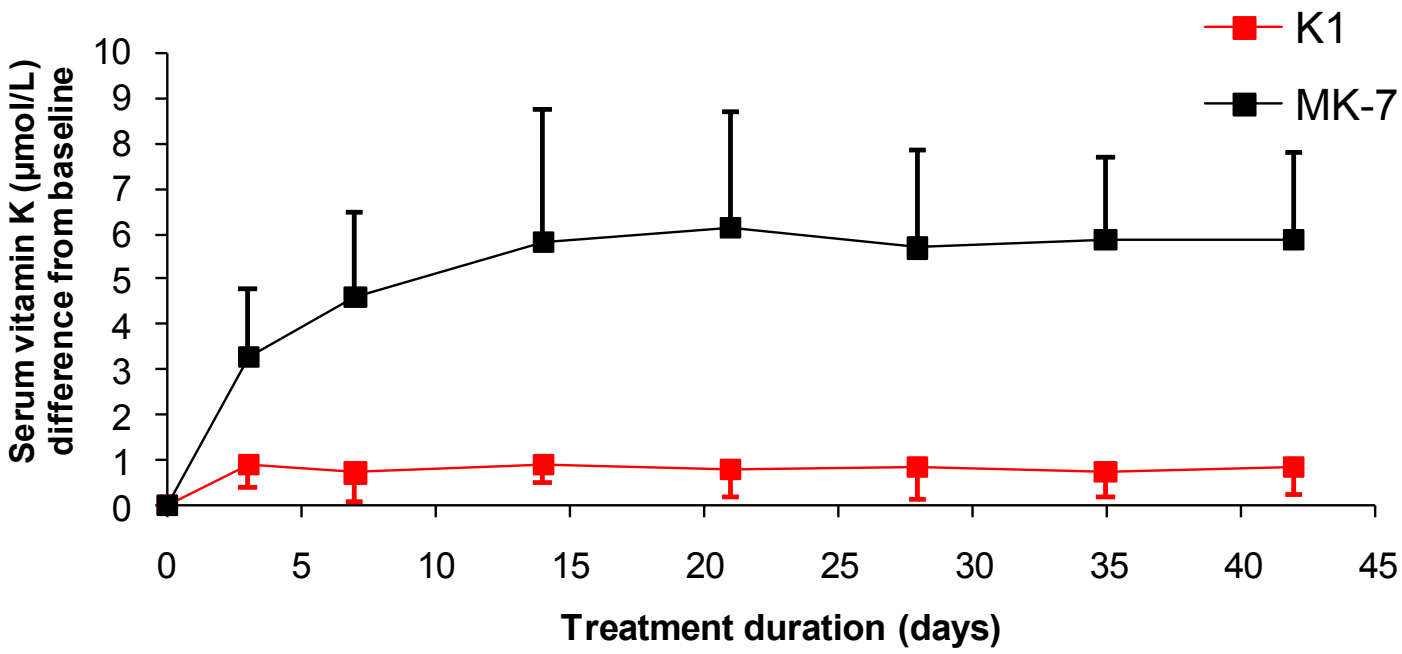

Prothrombin
FVII
FIX
FX
Protein C
Protein S
Protein Z
Intestine
(dietary vitamin K
from supplement)
Mixed
micelle
Lacteal
Thoracic duct
Capillaries
[Lipoprotein lipase]
CHYLOMICRONS
CHYLOMICRON REMNANTS
Blood circulation
Bone
VERY LOW-DENSITY LIPOPROTEINS (VLDL)
MK7 > MK4, K1
LDL
VLDL REMNANTS
MK7 > K1
Capillaries
[Lipoprotein lipase]
Matrix Gla-protein
Gas-6
Protein S
Gla-rich Protein

Function of vitamin K





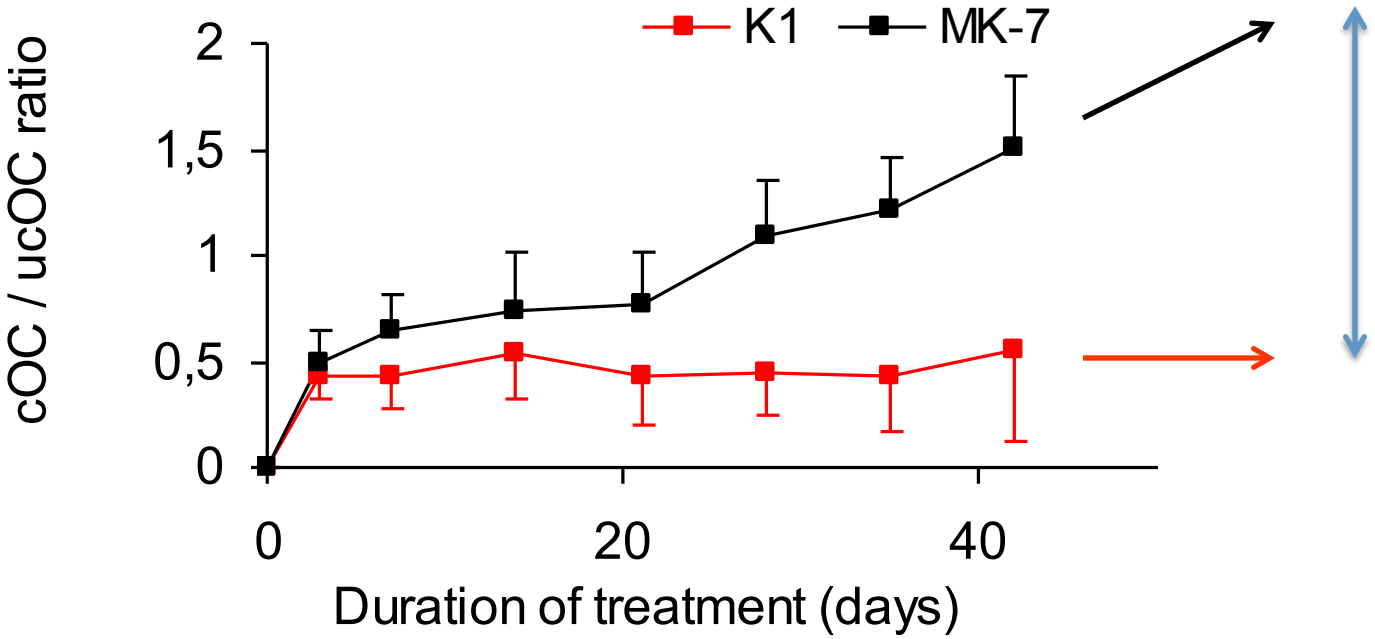
Accumulation during prolonged intake



- No accumulation of K_1 but significant accumulation of MK7
- After 14 days a steady level for MK-7 was reached
- Final level for MK-7 was 7-8 fold higher than for K_1

If taken on a daily basis, 45 µg/day of MK-7 is more effective than 240 µg/day of K_1 (twice the RDA!)

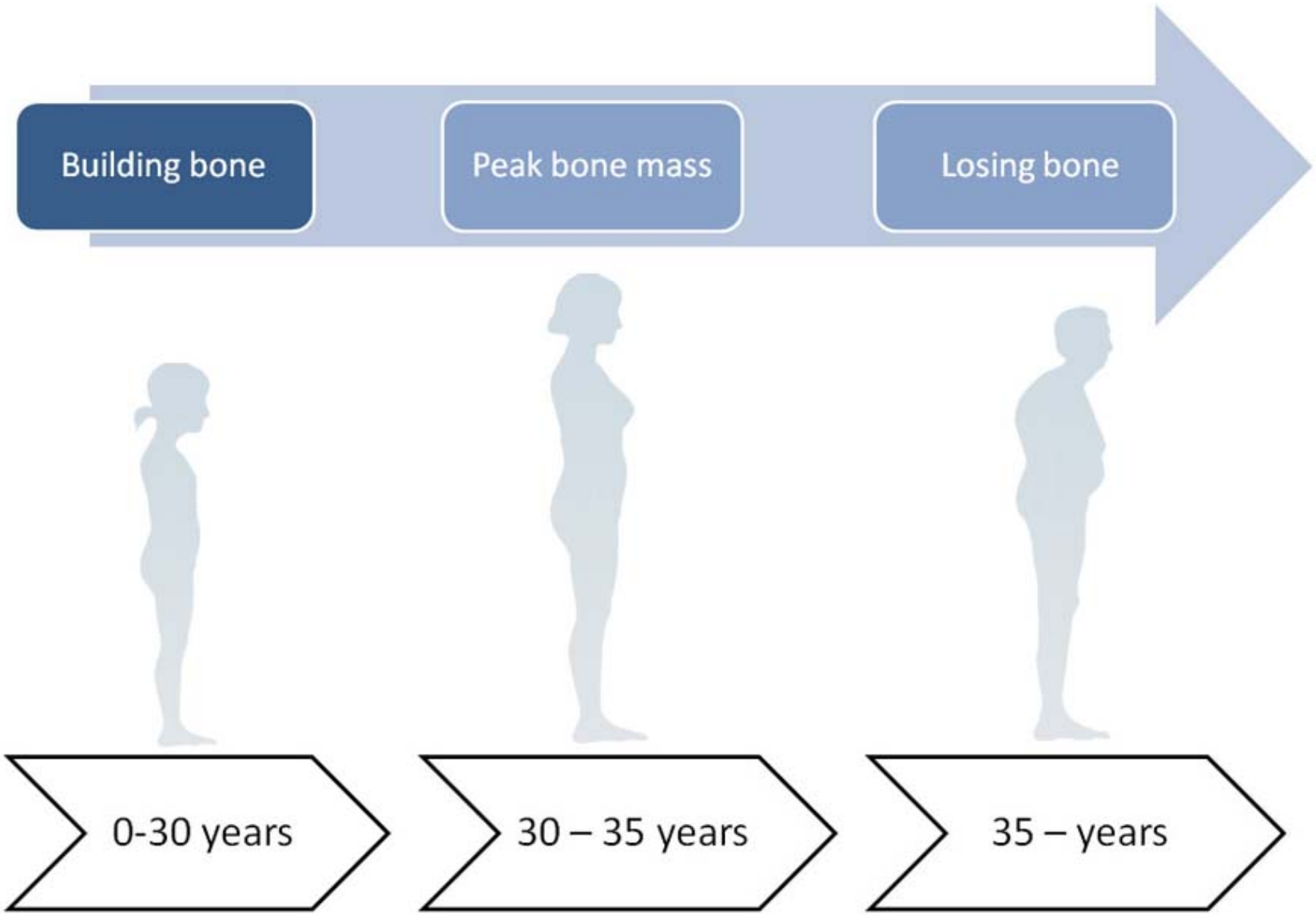
Schurgers et al. Blood 2007



- MK-7 is more effective than K_1 in improving vitamin K status → effect visible after 2-3 weeks
- Effect most pronounced after 6 week
- At that time MK-7 was over 3 times more effective than K_1

Schurgers et al. Blood 2007

Vitamin K and bone health

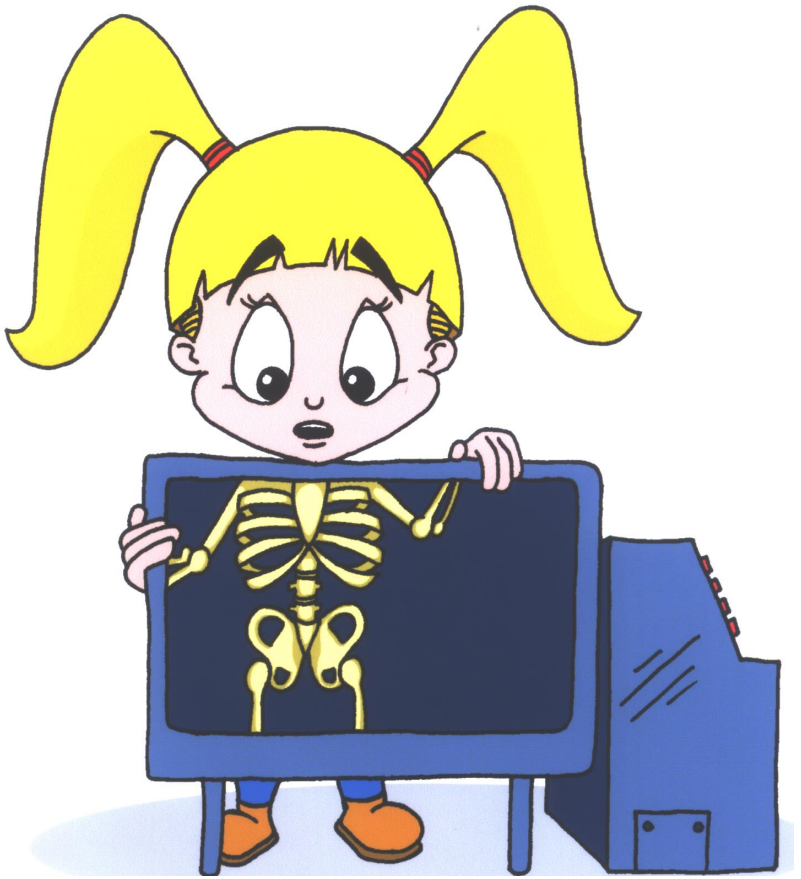


Why is K-status in childhood important?

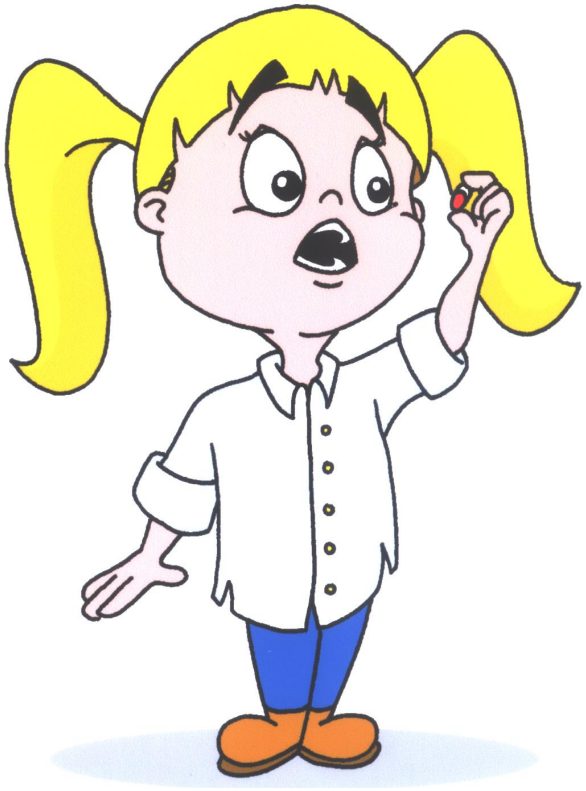
- The higher the “peak” bone mass (achieved at age < 30 years) the more you are protected to develope osteoporosis

- Young bone is highly active and osteocalcin levels are

  8 – 10 fold higher as compared to adults → requirement of vitamin K thus also higher



The Vita*K*ids study

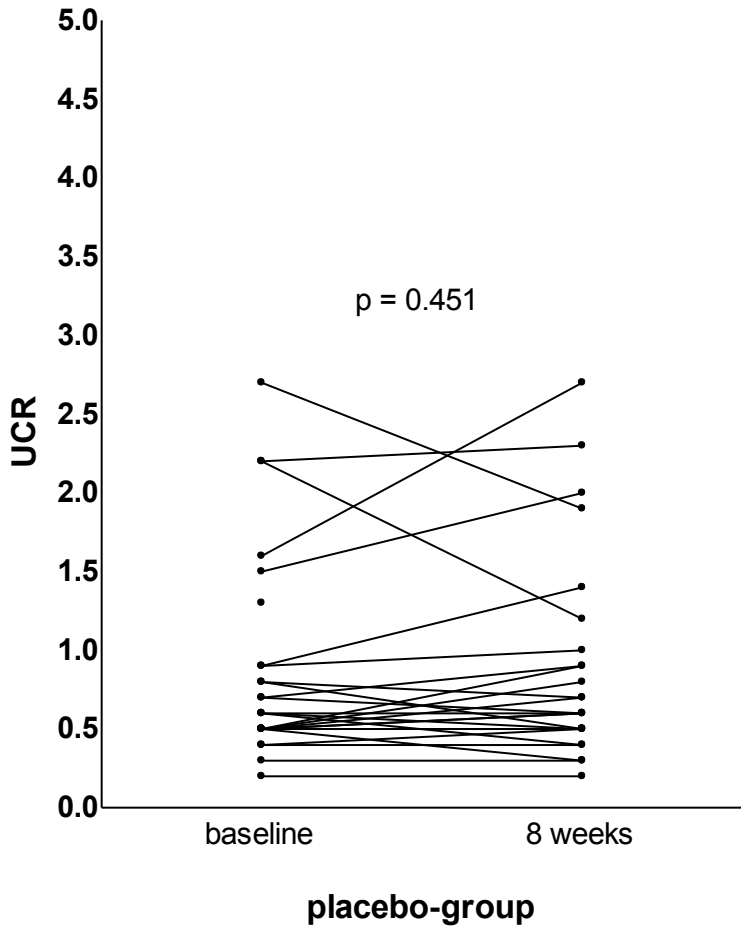


- Randomized, placebo controlled, double blind
- 60 children, 6-10 years of age
- Equal boys / girls
- 45 µg MK7 daily for 8 weeks
- Measurement of ucOC and cOC to assess the vitamin K-status

The effect of MK7 on
osteocalcin carboxylation in healthy children

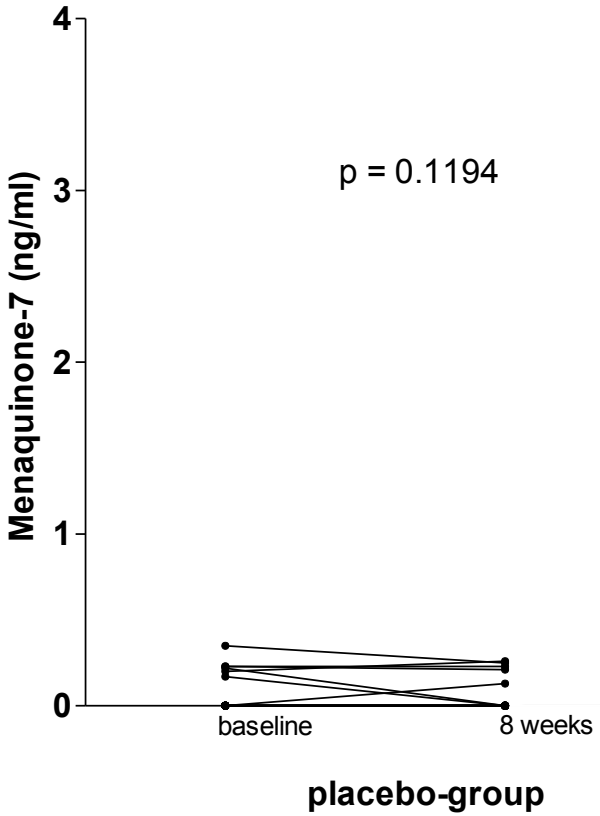
Summeren et al., BJN 2009

UCR: ucOC/cOC ratio



Summeren et al., BJN 2009

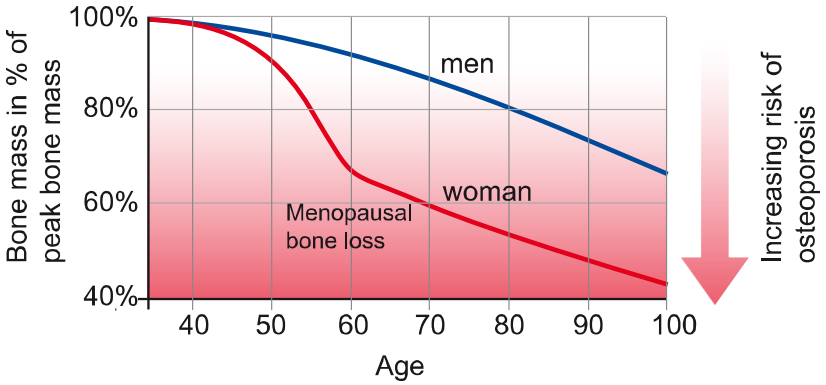
Menaquinone-7 absorption from MenaQ7

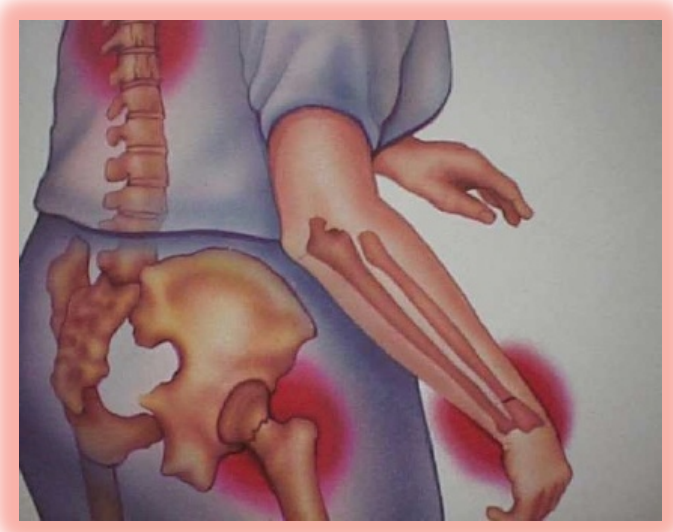


Summeren et al., BJN 2009

Osteoporosis

- Osteoporosis affects 75 millions in Europe, US and Japan
- Over 90 million people have osteoporosis in China
- 45% of women over 50 years will experience fractures, and 20 – 25% of men will suffer from fractures when over 50





Total cost* of fractures

US (2007)	EU (2003)
USD 18 billion	EUR 32 billion

Osteo-K2 study

- 325 postmenopausal women (55-75 years)
- Treatment for 3 years with daily supplementation

1. P group	Placebo
2. K2 group	**45 mg MK-4**

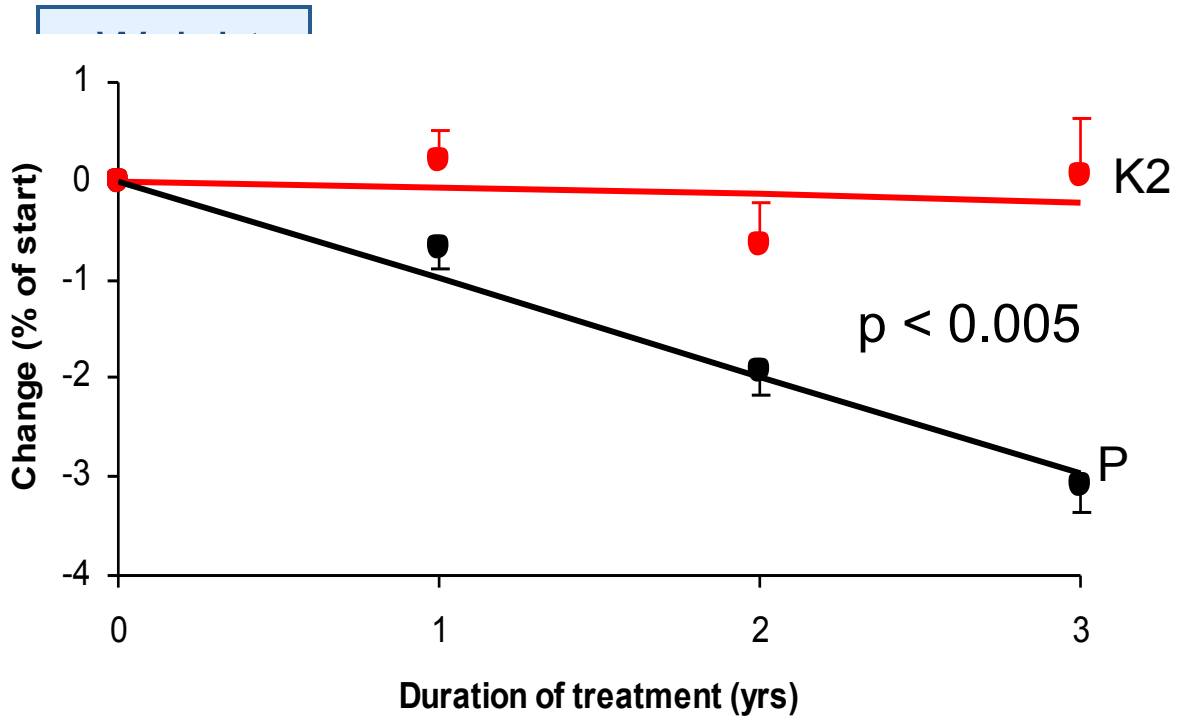
- Measurements:

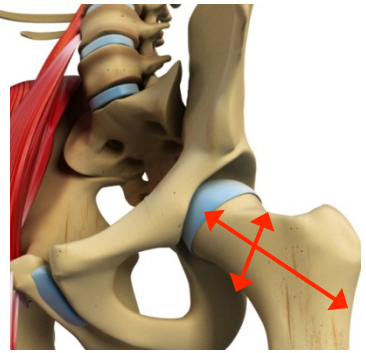
  BMD, bone mineral content (BMC), bone strength, and bone markers

Knapen et al., Osteoporosis Int 2007

Osteo-K2 study

- Bone strength





- **No loss of bone strength**

- **Improving BMC and FNW**
- **Improving ucOC levels**

Knapen et al., Osteoporosis Int 2007

Osteo-MK7 study

- 240 postmenopausal women (55-65 years)
- Treatment for 3 years with daily supplementation

1. P group	Placebo
2. MK-7 group	**180 µg MK-7** (MK-7)

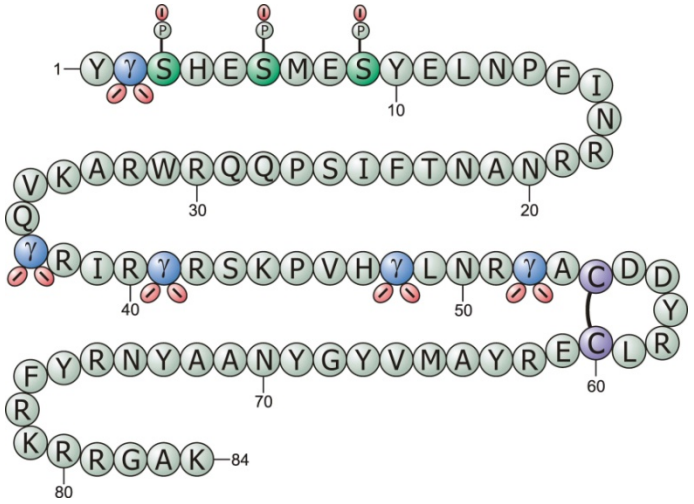
- Measurements:
  - ✓ Bone health:
    BMD, BMC, bone strength, bone geometry, and bone markers
  - ✓ Vascular health:
    IMT, distensibility & elasticity of carotid artery, PWV

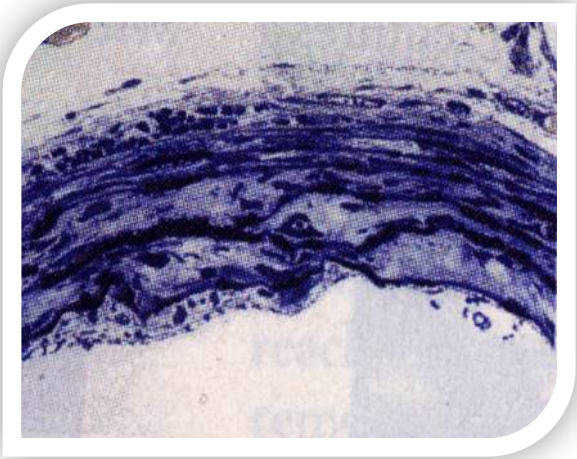
Vascular disease and vascular calcification

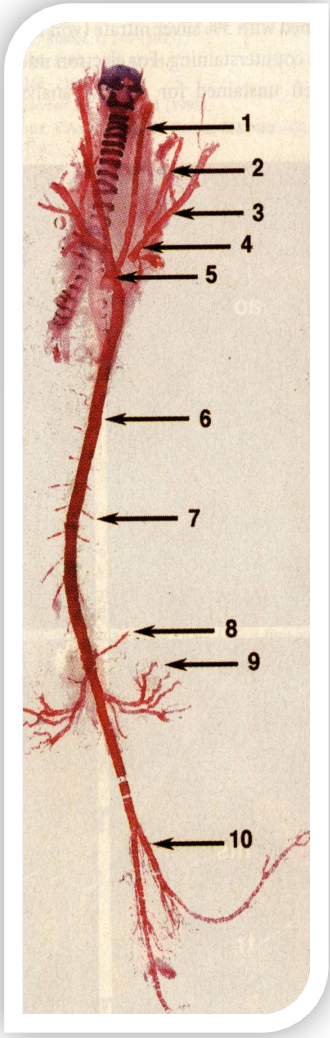
Precipitation of calcium-salts at pathological sites

- Hypercalcemia (>2.8mM)
- Hyperphosphatemia (>2.0mM)
- Atherosclerosis (Ca> 30mM)
- End-Stage Renal Disease (Ca x P)
- Hypertension (intracellular Ca-overload)

Matrix Gla-protein (MGP): the vascular calcification inhibitor in need of vitamin K







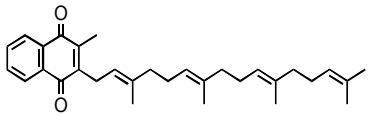
- vitamin K-dependent protein
- 84 amino acids (Mw ~11 kD)
- Gla-residues (required for activity)
- Serine-phosphorylation (function unknown)

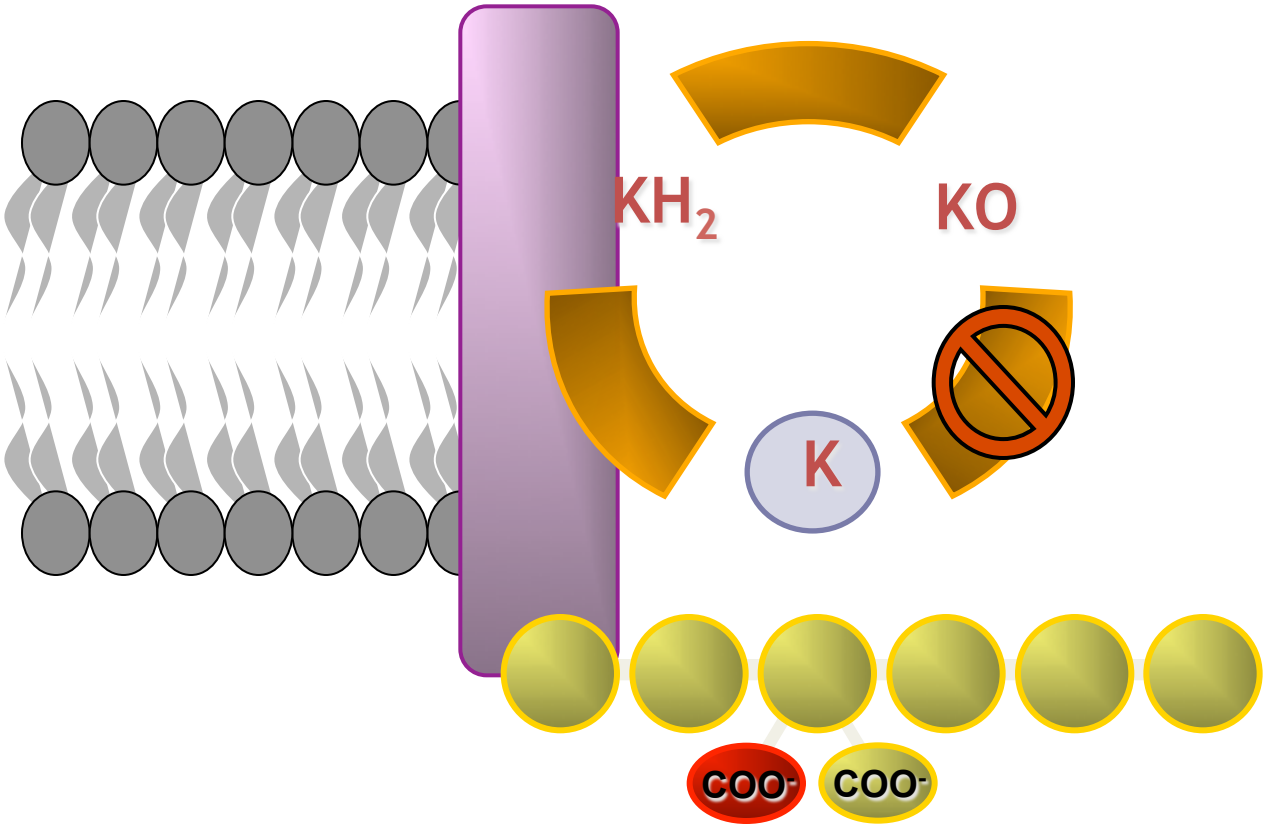
Luo et al. Nature 1997

VKA: from rat poison to drug

- 1925, rare cattle disease
- 1948, dicoumarols launched as rat poison
- 1951, unsuccessful suicide attempt by US army soldier with warfarin
- 1954, warfarin approved as medicine
- 1955, Eisenhower one of the first famous recipient because of an heart attack

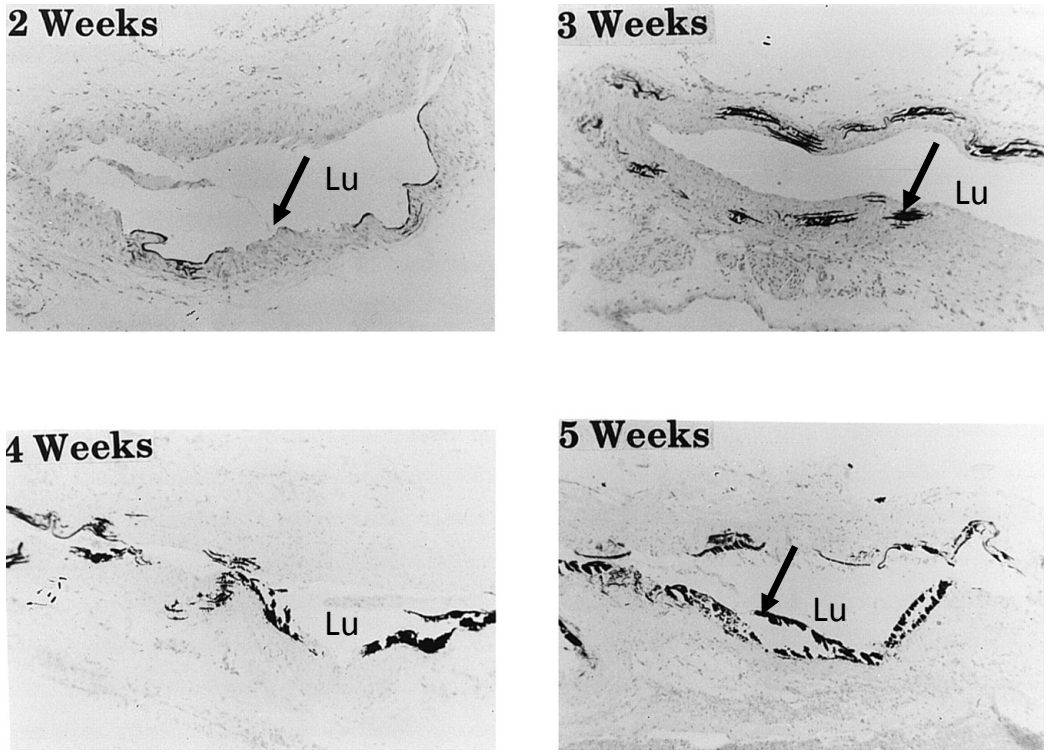
Interference with vitamin K-metabolism





Warfarin Causes Rapid Calcification of the Elastic Lamellae in Rat Arteries and Heart Valves

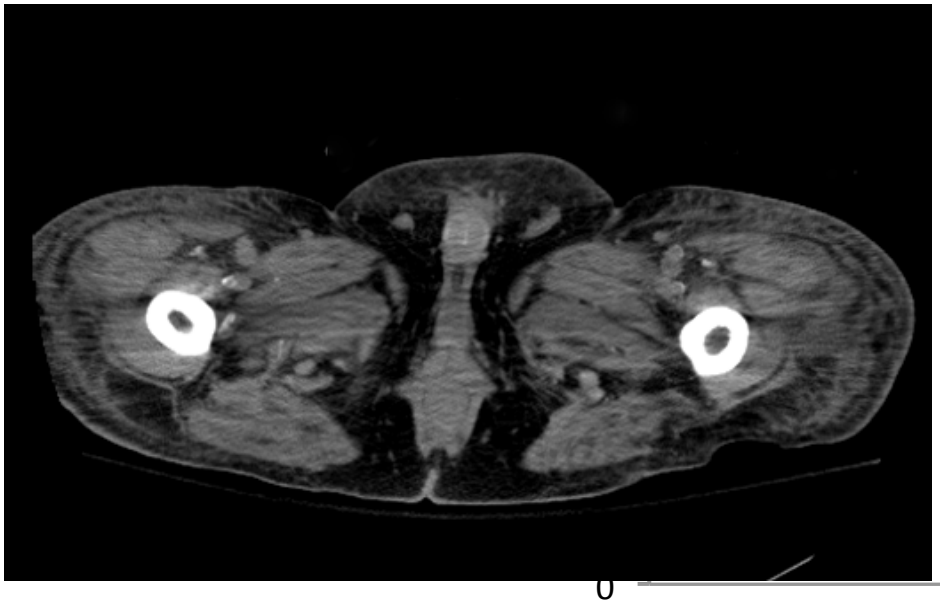
Paul A. Price, Samuel A. Faus, Matthew K. Williamson

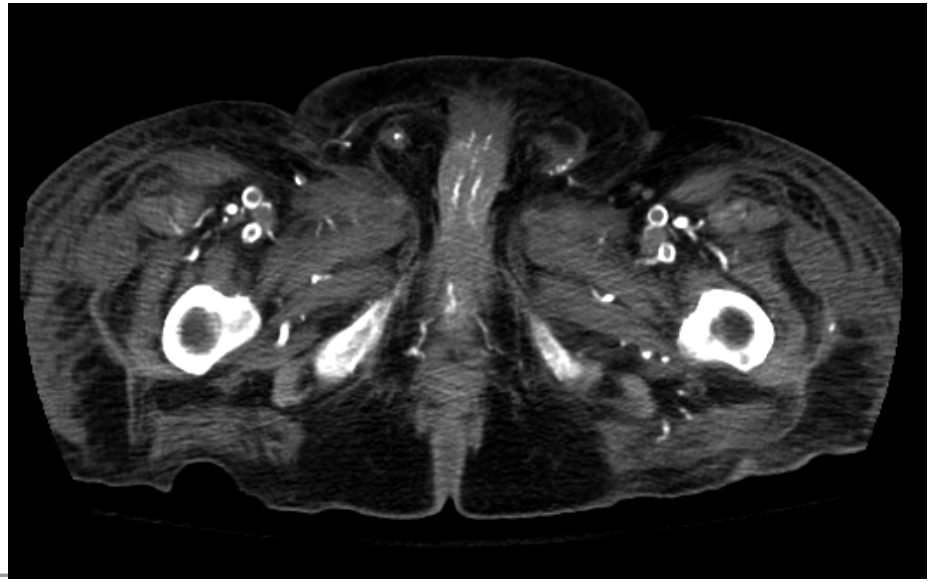


Warfarin induced artery calcification is promoted by increases in serum calcium or phosphate. Strong upregulation of MGP at sites of calcification, though in the inactive uncarboxylated form

Price et al. ATVB 1998

Warfarin induced calcifications





CT of patient before coumarin treatment

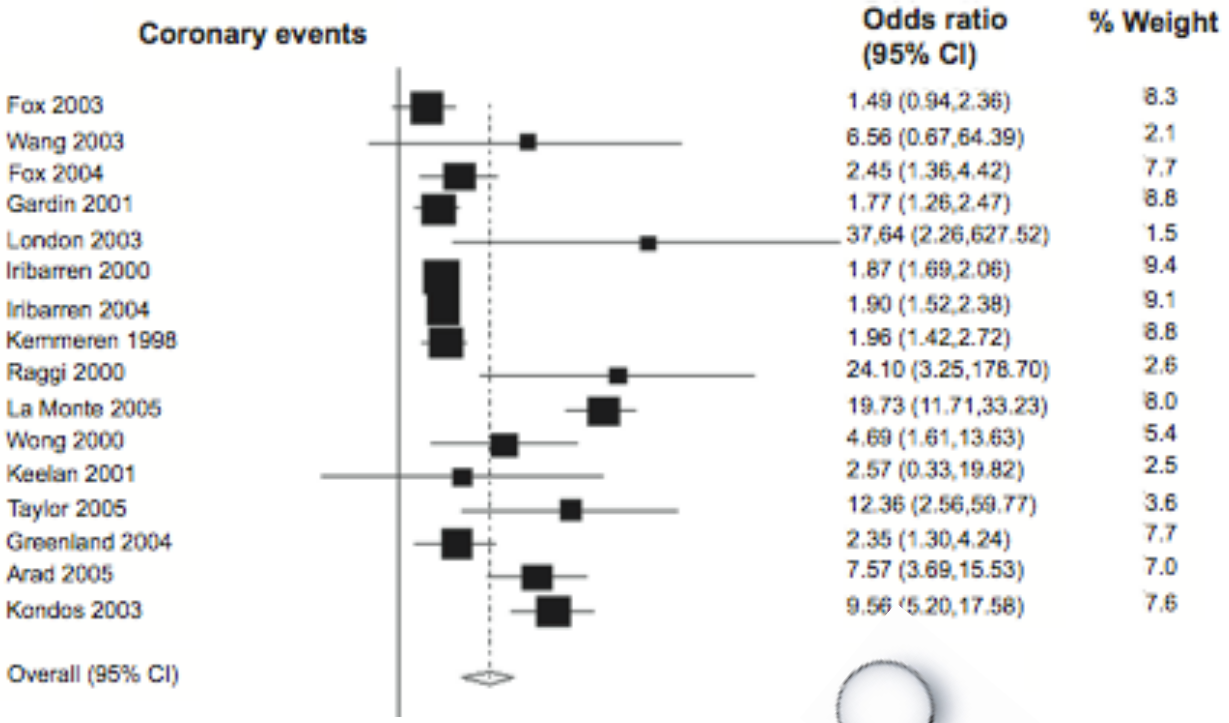
CT of patient 9 months after start of coumarin treatment

Schurgers et al. Blood, Nov 2004

Variable	Oral anticoagulants		P value
Agatston score	Yes (n =23)	No (n = 63)	
Valve	2,410	1,070	0.003
Coronary	1,561	738	0.024

Koos et al. JACC 2005

Vascular calcification as a marker of increased cardiovascular risk: a meta-analysis



Rennenberg et al. Vascular health and risk management 2009

Coronary artery calcium is a better predictor of cardiovascular events than the Framingham risk score and can help to reclassify asymptomatic individuals into high-risk or low-risk categories

Alexopoulos et al. Nature Reviews Cardiology 2009

Requirements for mineralization

BONE

VASCULATURE

INITIATION

matrix vesicles / apoptotic bodies

apoptosis and matrix vesicles

loss of inhibitors

MGP

NUCLEATION

osteoblast matrix

elaboration of calcifying matrix
source of Ca and P

CRYSTAL GROWTH

osteoblastic conversion of VSMCs

osteoclasts

osteoclast-like macrophages?

REABSORPTION

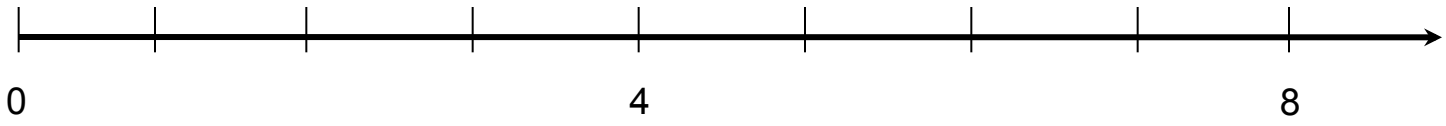
Influence of VKA on medial calcification

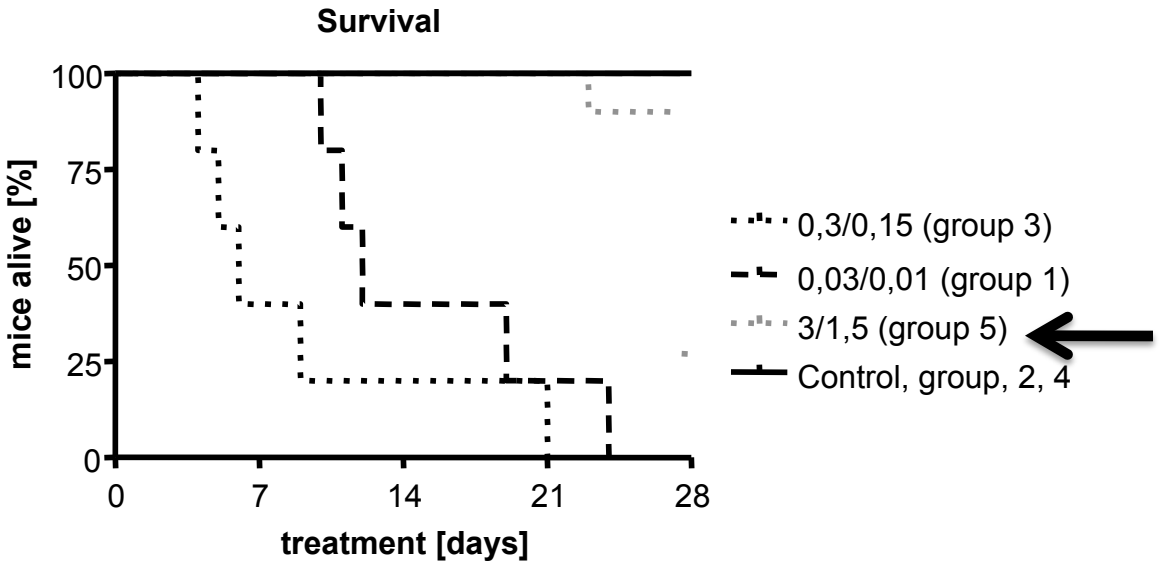
Control diet

0,03 mg Warfarin / 1,5 mg K1

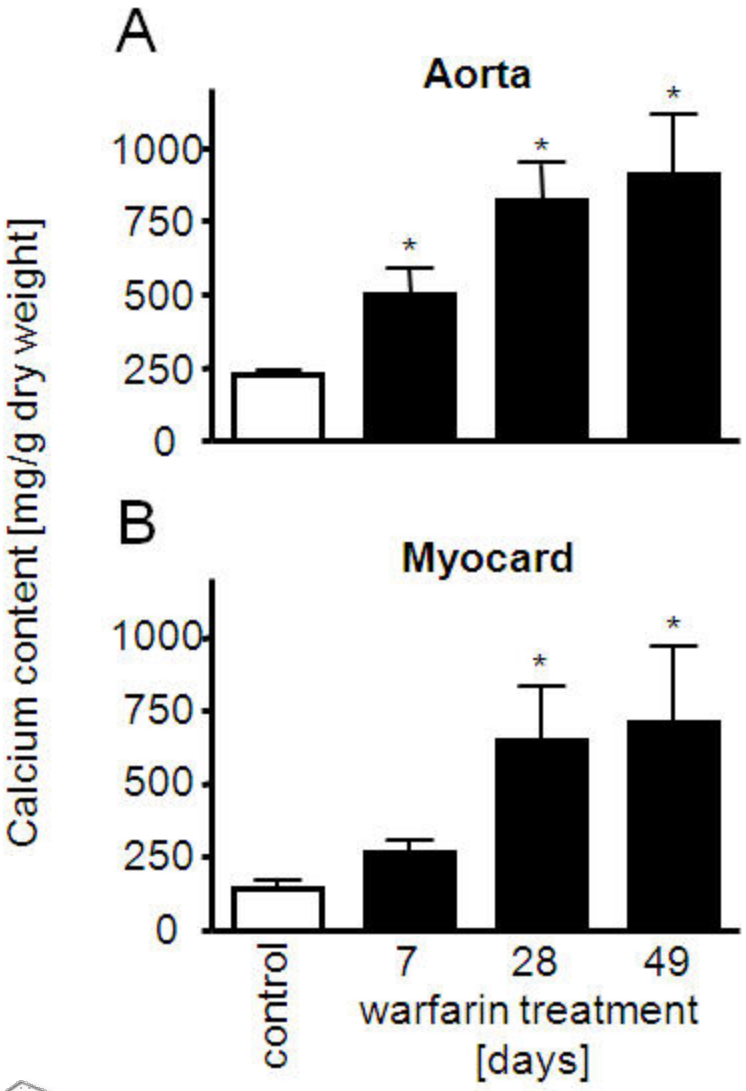
0,3 mg Warfarin / 1,5 mg K1

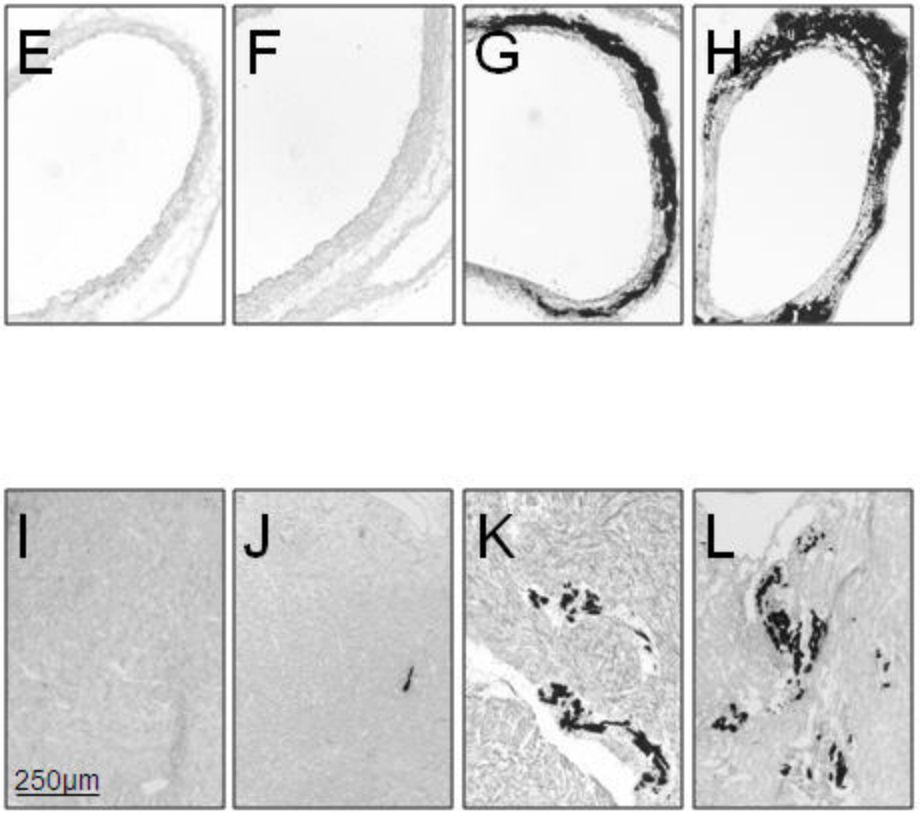
3 mg Warfarin / 1,5 mg K1





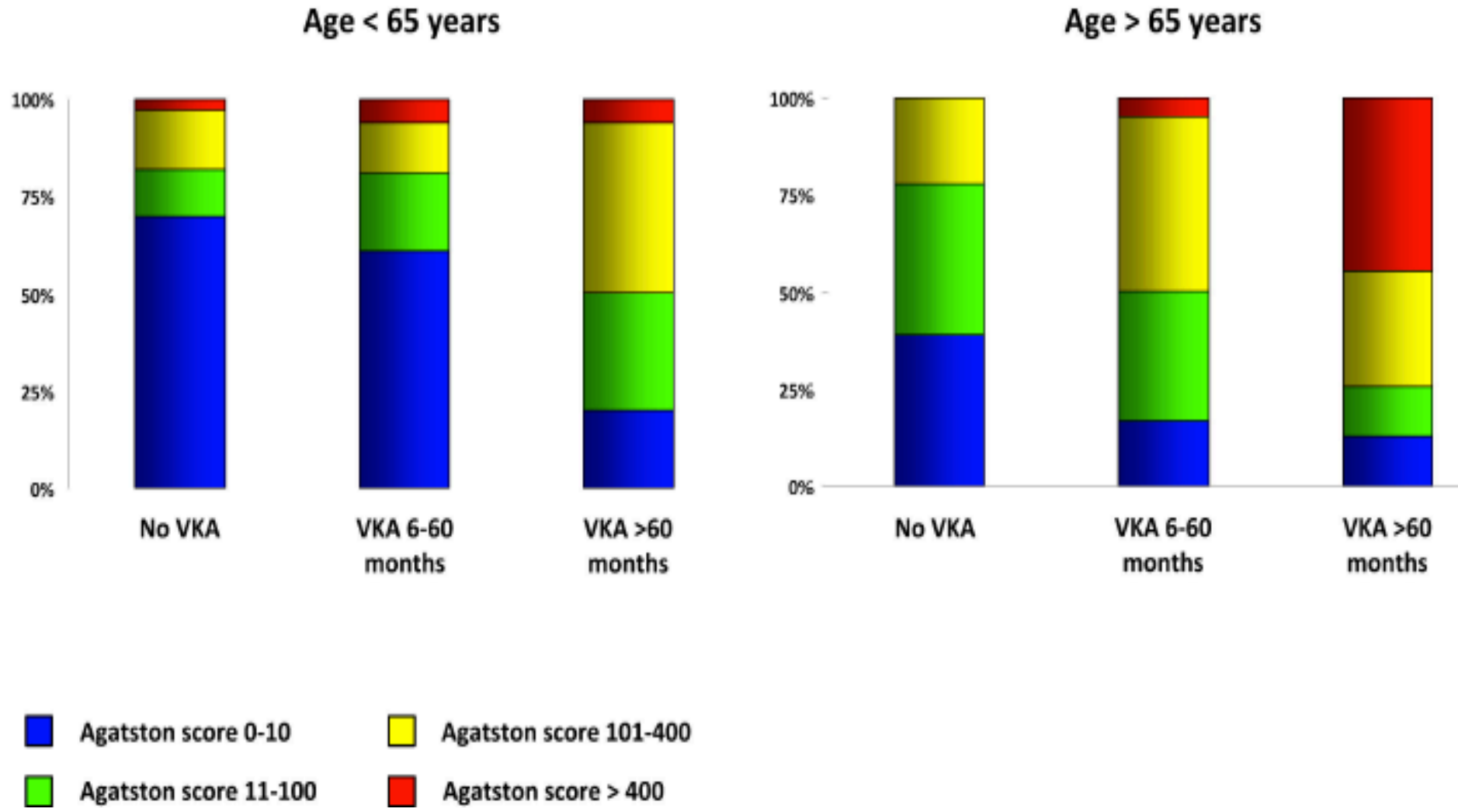
Influence of VKA on medial calcification: time dependency



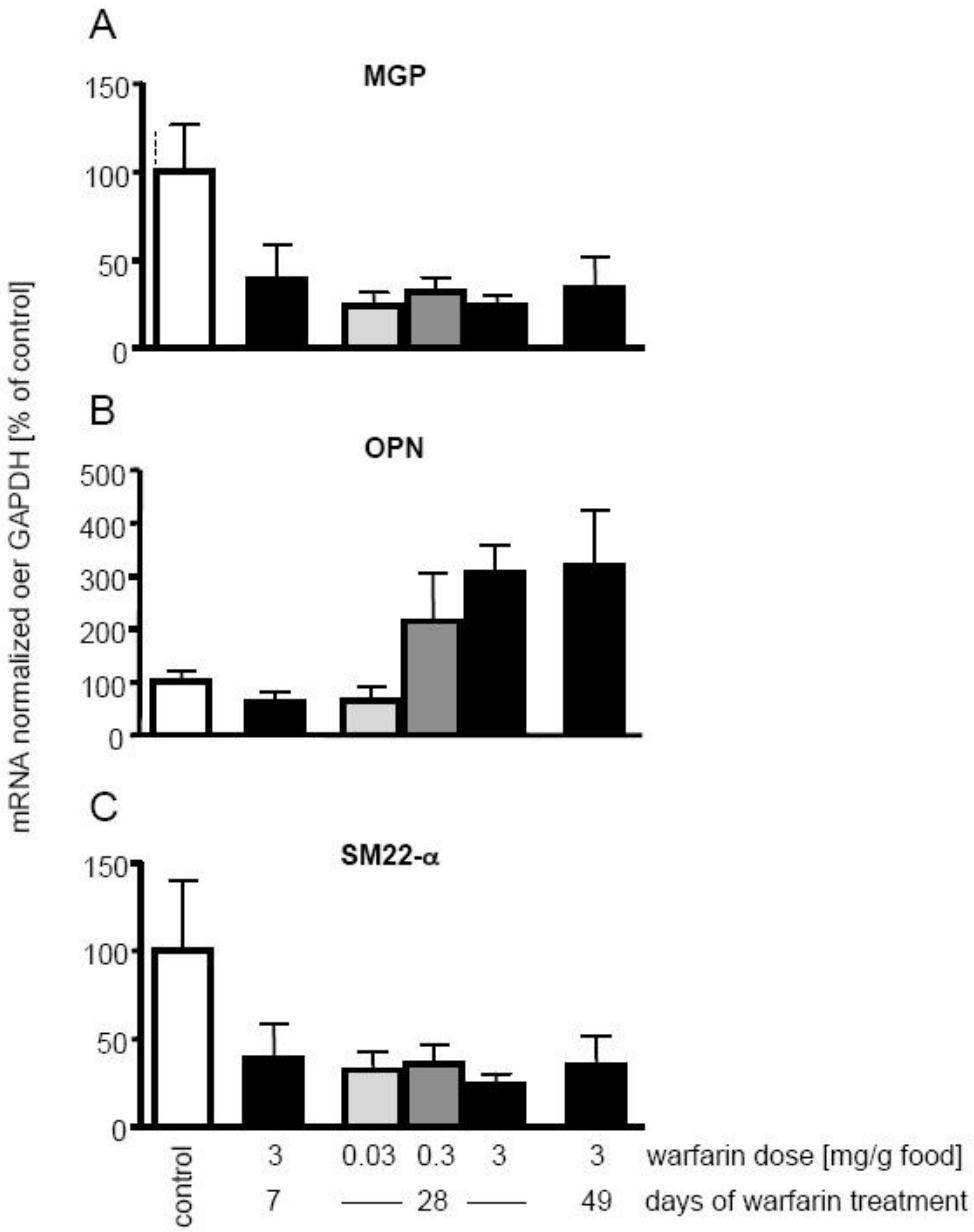


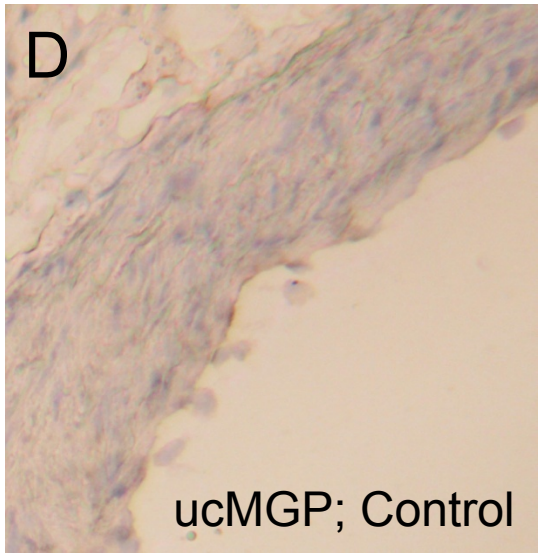
Submitted

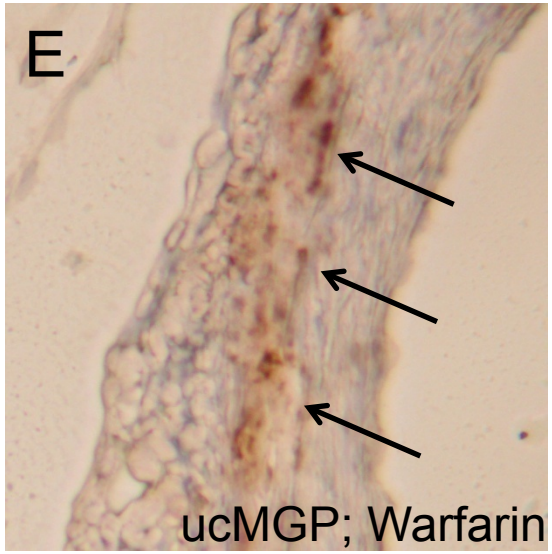
Low AF patients on warfarin treatment



Weijs et al. Eur Heart J 2011


A
MGP
150
100
50
0
B
OPN
500
400
300
200
100
0
C
SM22-α
150
100
50
0
mRNA normalized oer GAPDH [% of control]
control
3
0.03
0.3
3
3
warfarin dose [mg/g food]
7
28
49
days of warfarin treatment


D
ucMGP; Control


E
ucMGP; Warfarin

Submitted

“Clinical” consequences of VKA in mice

	Control	28 days warfarin	49 days warfarin
Aortic valve Peak Gradient [mmHg]	8.6 ± 2.5	8.6 ±3.8	25.9 ±3.1
Aortic stiffness pulse wave velocity [m/s]	1.9 ± 0.25	2.8 ± 1.44	4.8 ± 0.47

Oral anticoagulation results in medial vascular calcification, associated with vascular smooth muscle cell loss and parameters of vascular stiffening

Diet composition:

1) **Control diet**

3 mg Warfarin / 1,5 mg K1

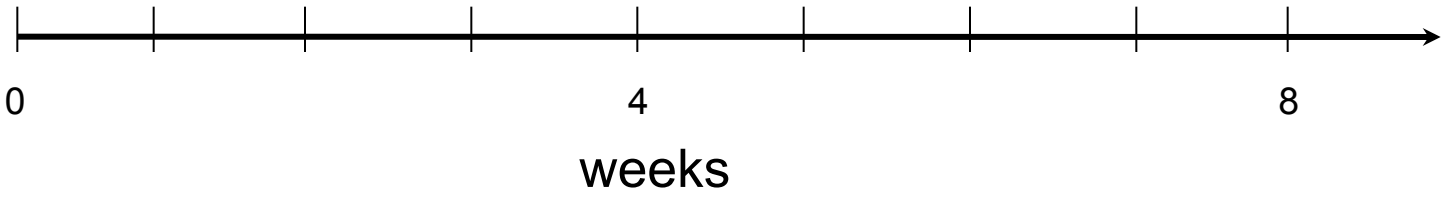
Blocking vit K

2) **3 mg Warf. /** 1,5 mg K1 / **10 µg MK-7**

Inhibition by K2

3) **3 mg Warfarin /** 1,5 mg K1 | **10 µg Vitamin MK-7**

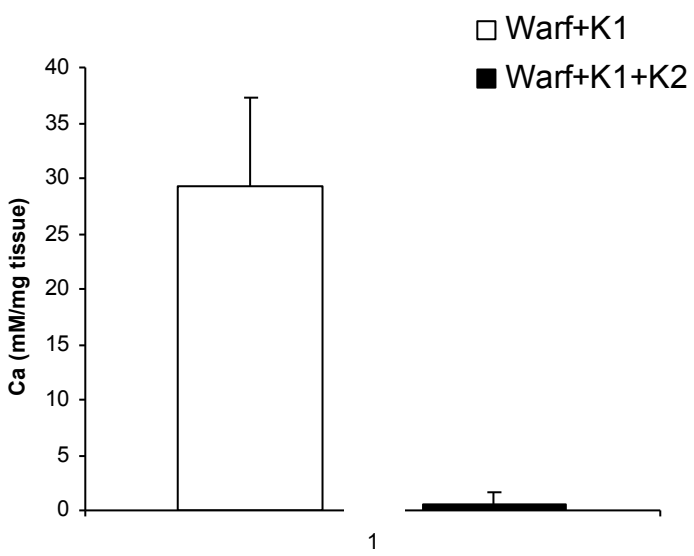
"Therapy"



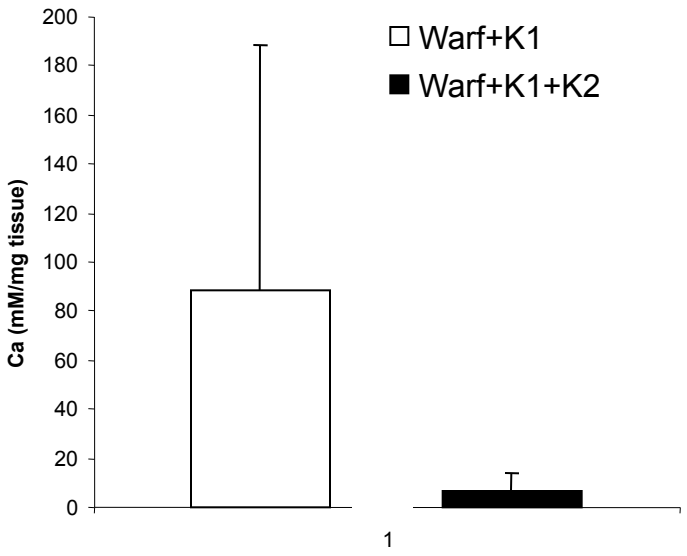
Krueger, Westenfeld, Schurgers

Simultaneous administration of Vitamin K2 (MK7)

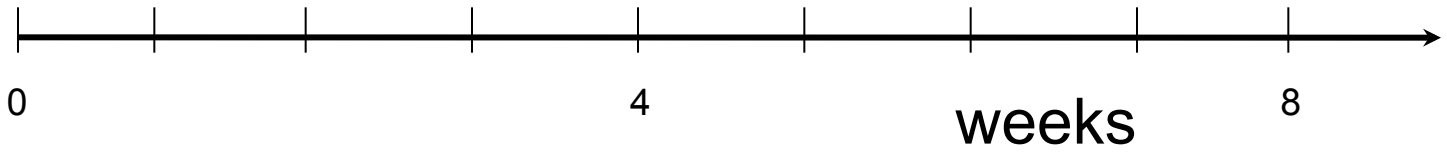
Ca content in aorta



Ca content in myocardium

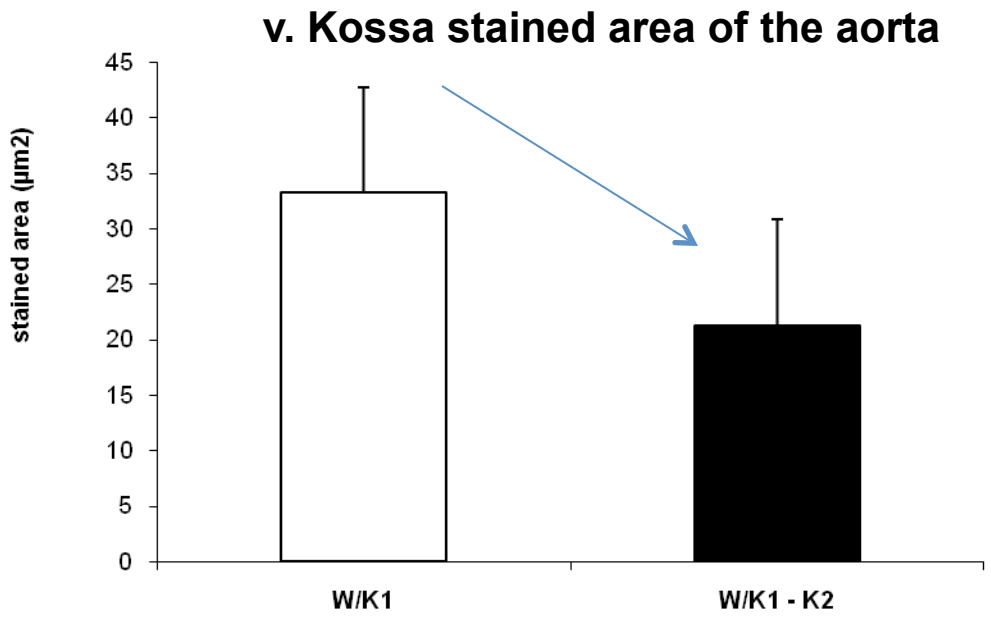


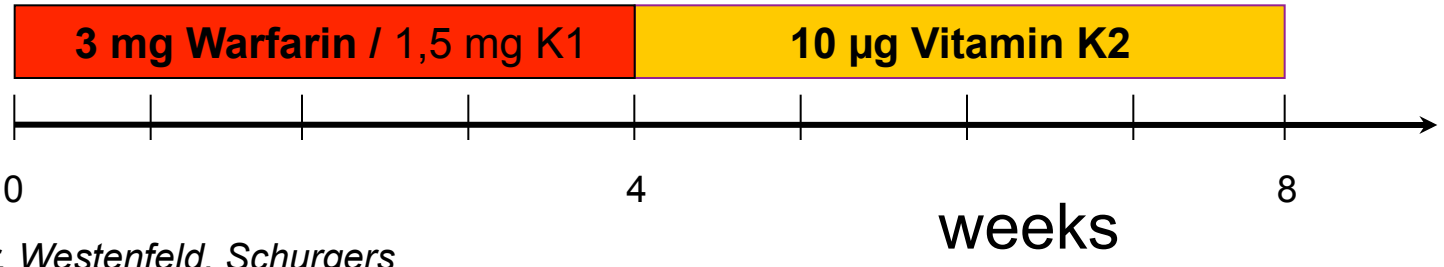
3 mg Warf. / 1,5 mg K1 / **10 µg K2**



Krueger, Westenfeld, Schurgers

Therapy by sequential administration of Vitamin K2 (MK-7)



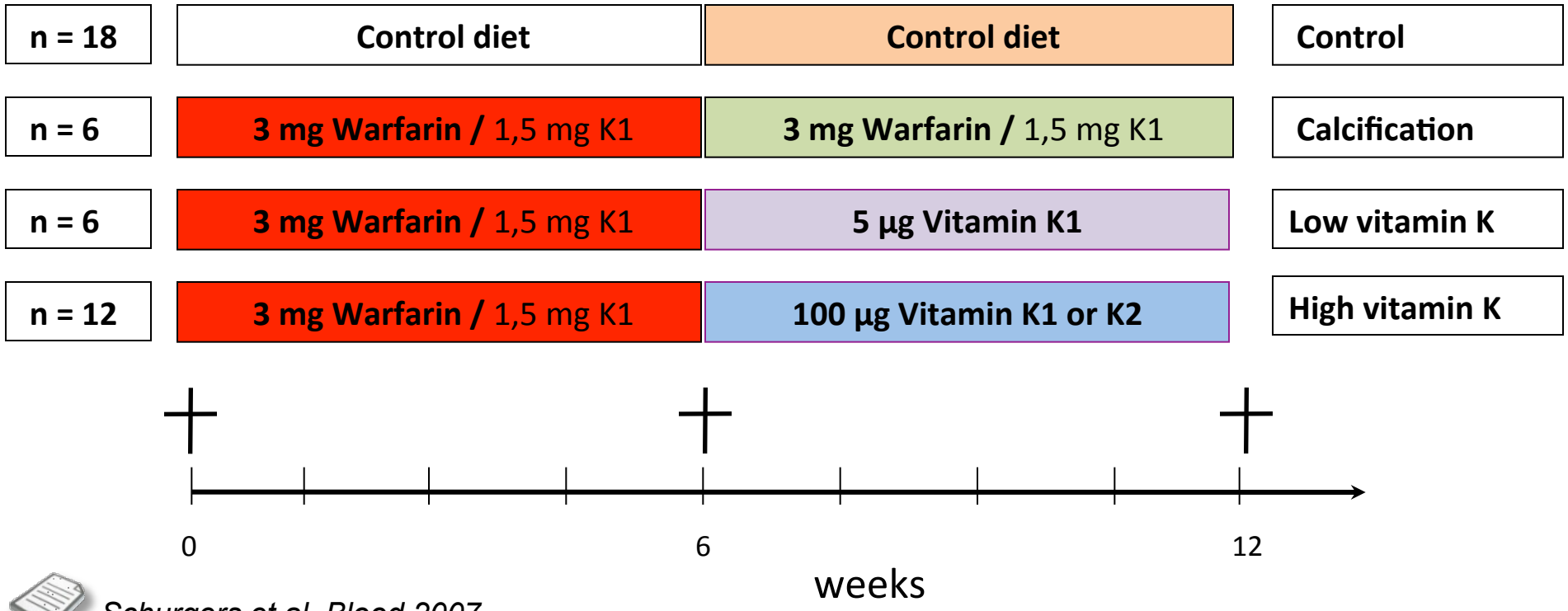


Krueger, Westenfeld, Schurgers

Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats

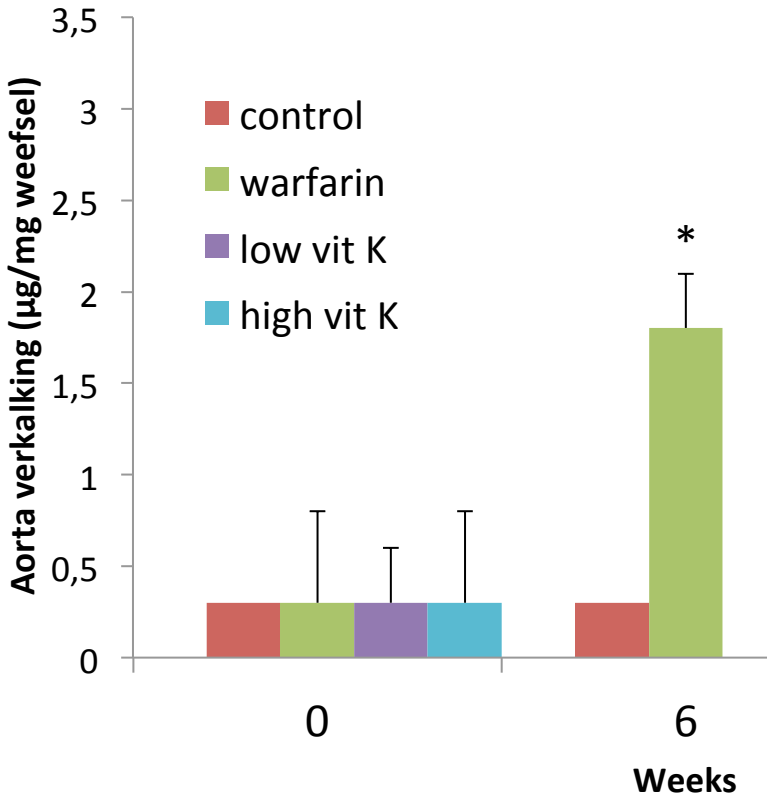
Leon J. Schurgers,[1,2] Henri M. H. Spronk,[3] Berry A. M. Soute,[1] Paul M. Schiffers,[4] Jo G. R. DeMey,[4] and Cees Vermeer[1,2]

[1]Cardiovascular Research Institute (CARIM), Maastricht University, The Netherlands; [2]VitaK, Maastricht University, The Netherlands; [3]Department of Internal Medicine, Cardiovascular Research Institute (CARIM), Maastricht University, The Netherlands; [4]Department of Pharmacology and Toxicology, Cardiovascular Research Institute (CARIM), Maastricht University, The Netherlands



Schurgers et al. Blood 2007

Can we stop or even *regress* pre-formed medial artery calcification?



37% reduction

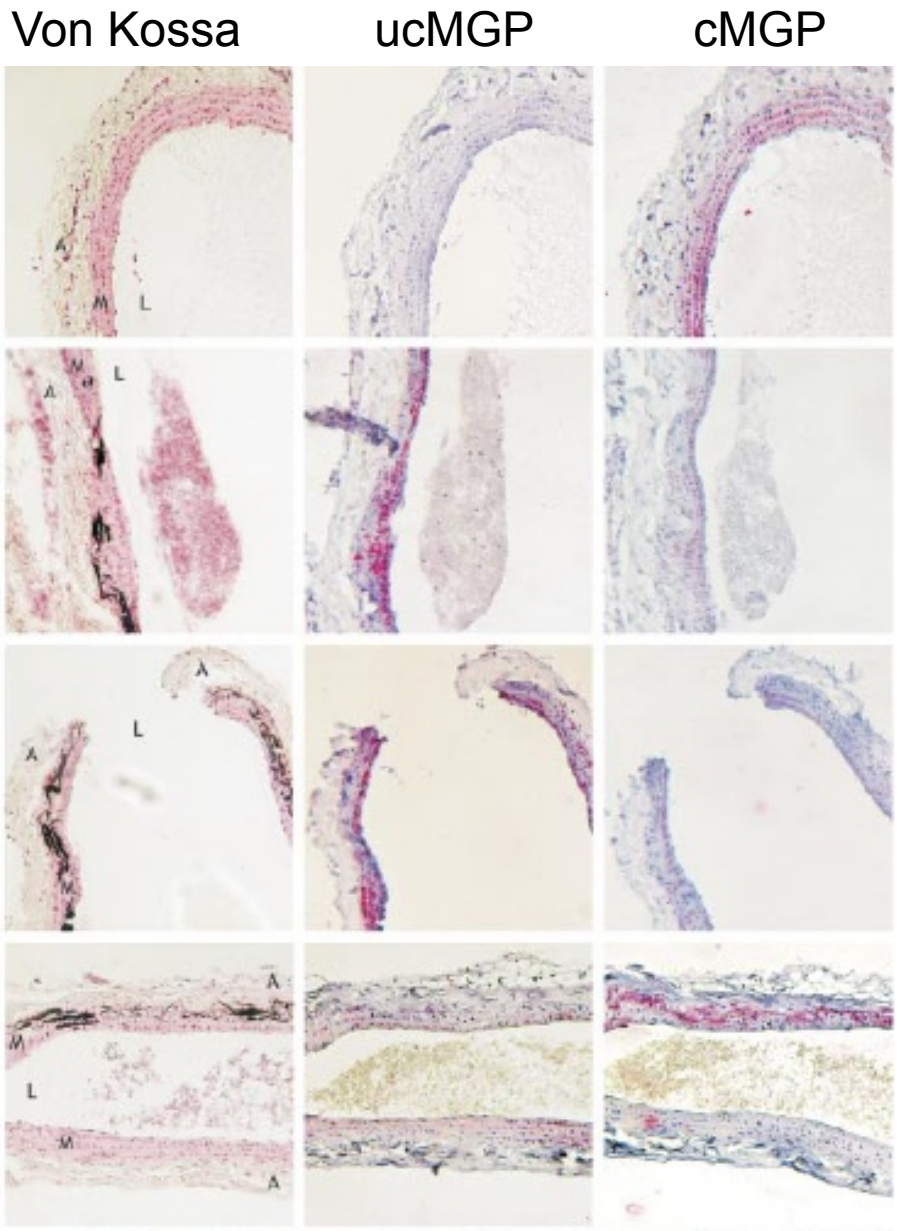
Schurgers et al. Blood 2007

Control diet

Warfarin / 1,5 mg K1

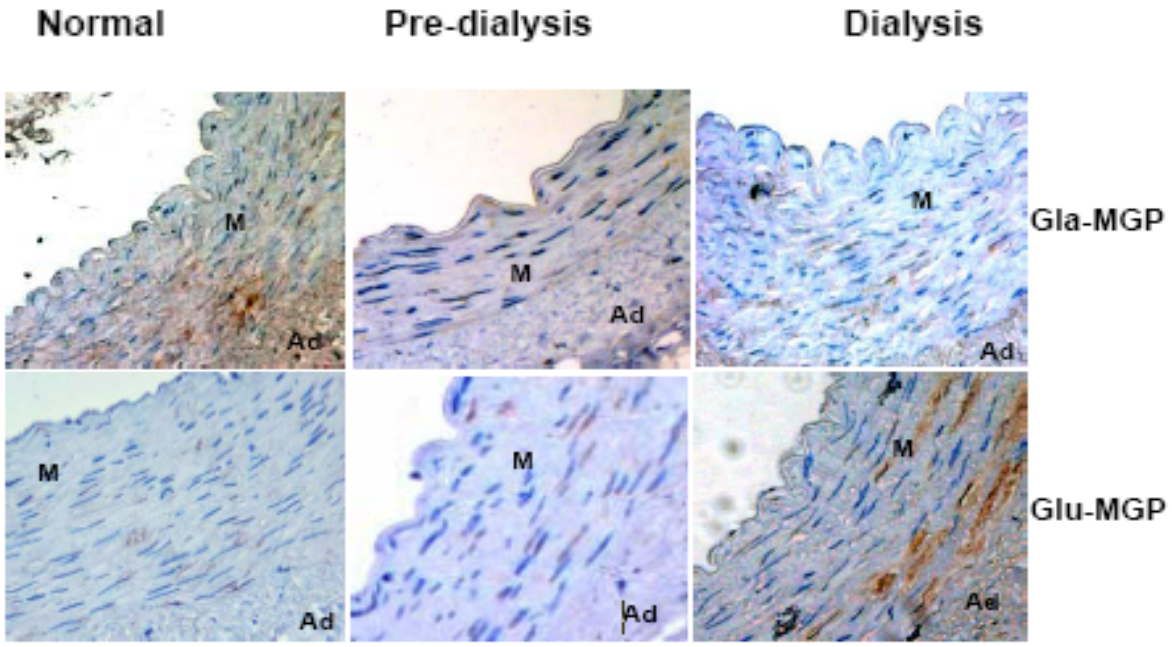
low Vitamin K

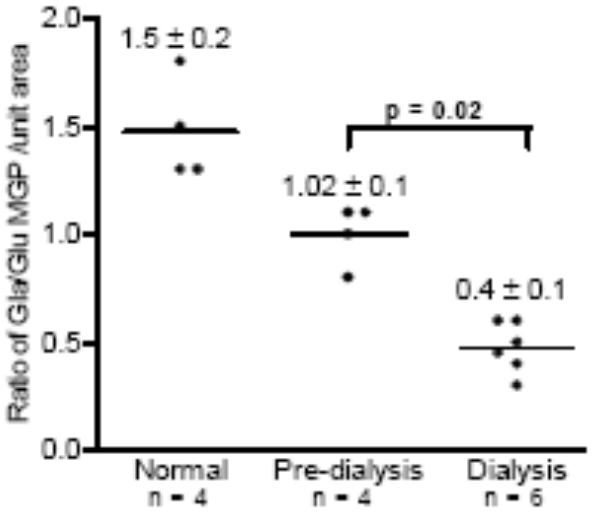
high Vitamin K



Schurgers et al. Blood 2007

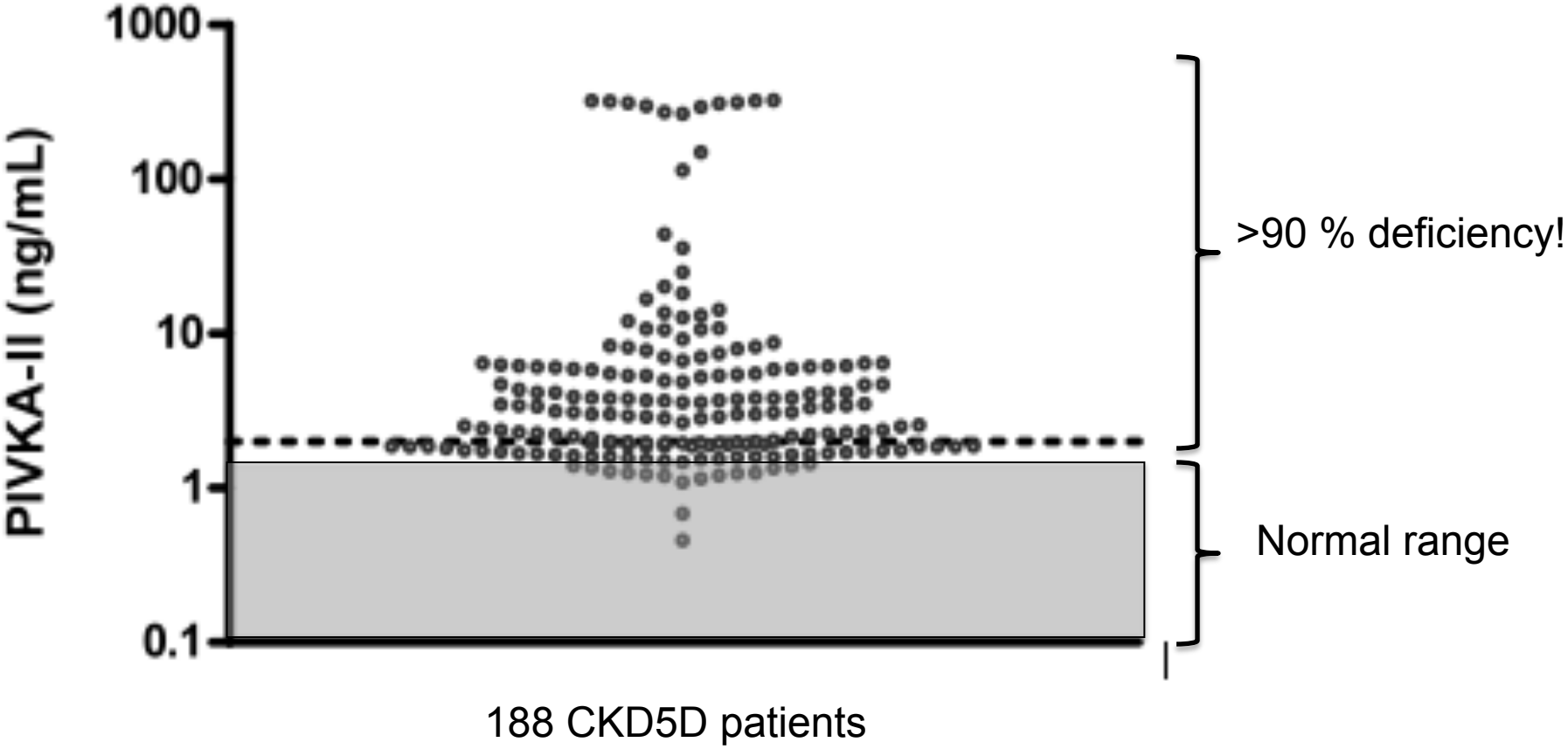
vascular calcification is triggering by smooth muscle cell vitamin K-deficiency





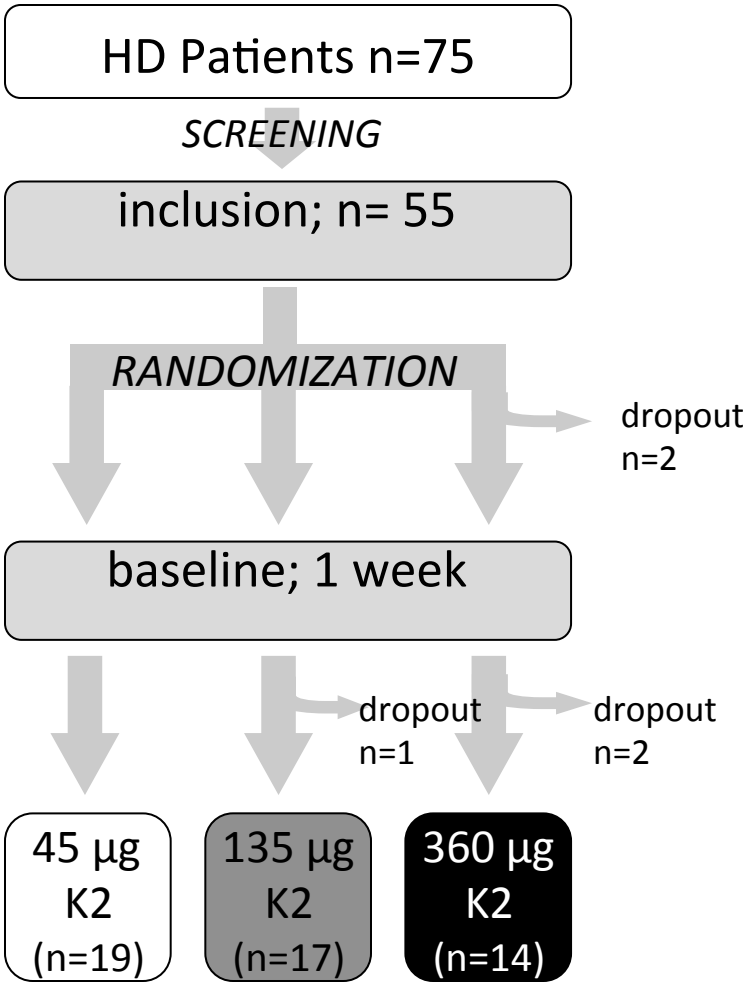
Shroff et al.Circulation 2008

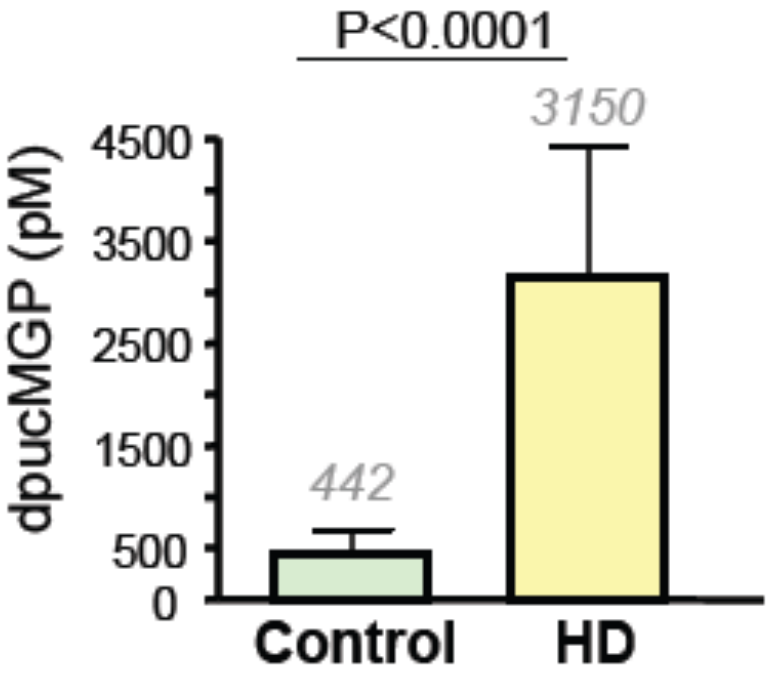
Vitamin K-deficiency in dialysis



Schlieper, JASN 2011

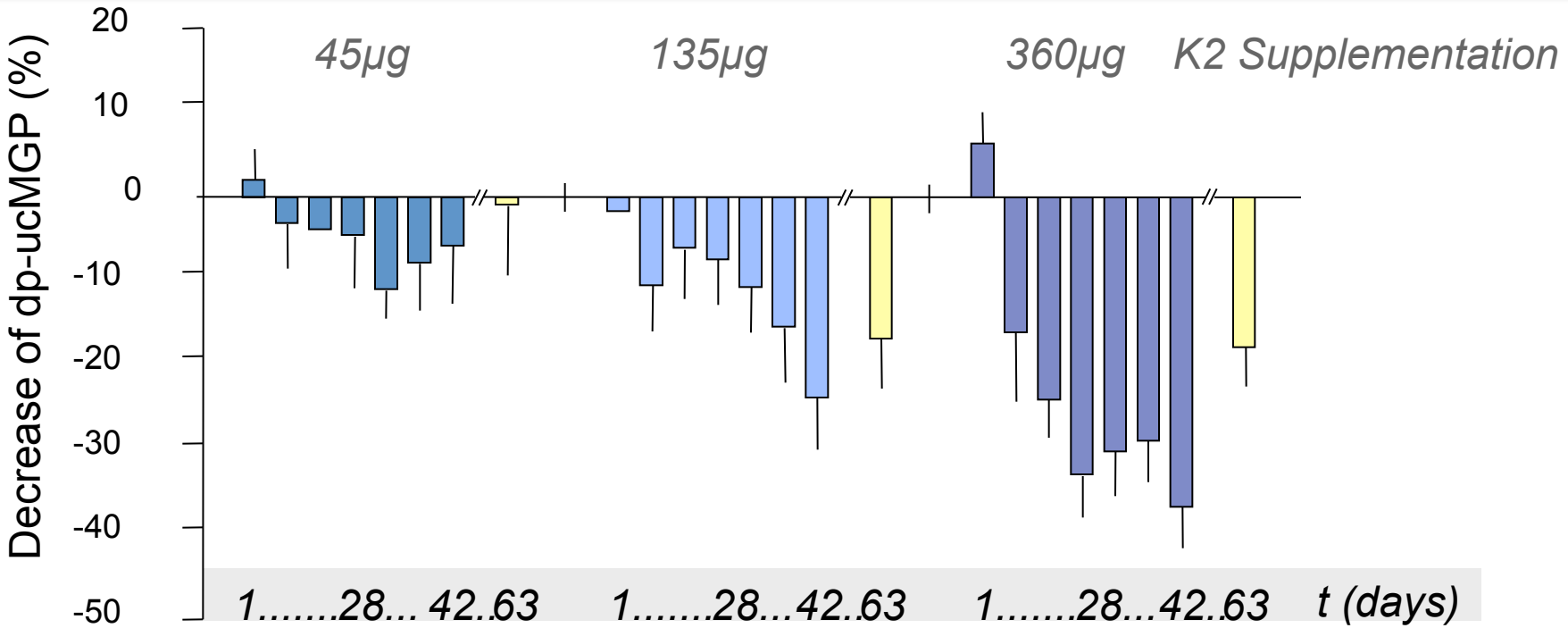
Vitamin K supplementation in dialysis patients





measurement of dp-ucMGP to show improved carboxylation in the vessel wall by high vitamin K intake

Submitted



- Vitamin K2 supplementation at 135µg and 360µg per day induces incremental reduction of dp-ucMGP levels over 6 weeks.
- Following termination of vitamin K2 supplementation dp-ucMGP levels rise again.

Submitted

EUROPEAN RENAL ASSOCIATION
EUROPEAN DIALYSIS AND TRANSPLANT ASSOCIATION
Registered Charity n° 1060134

VitaVasK-Study
- Design -

Population

- HD-patients (n = 650)
- Not on VKA
- CAC score > 30

randomised (1:1)

Standard therapy + placebo (n = 165)

Standard therapy + Vitamin K2 (MK7) (n =165)

follow-up = 1.5 years

Week 0

Week 40

Week 78

- End points: primary = progress of coronary calcification
  secondary = progress of aortic(-valve) calcification

VitaK-CAC Study
- Design -

Population

- CAC-patients (n = 200)
- Not on VKA
- CAC score >100; < 400

randomised (1:1)

Standard therapy + placebo (n = 100)

follow-up = 2.0 years

Standard therapy + Vitamin K2 (360mcg) (n =100)

Week 0

Week 52

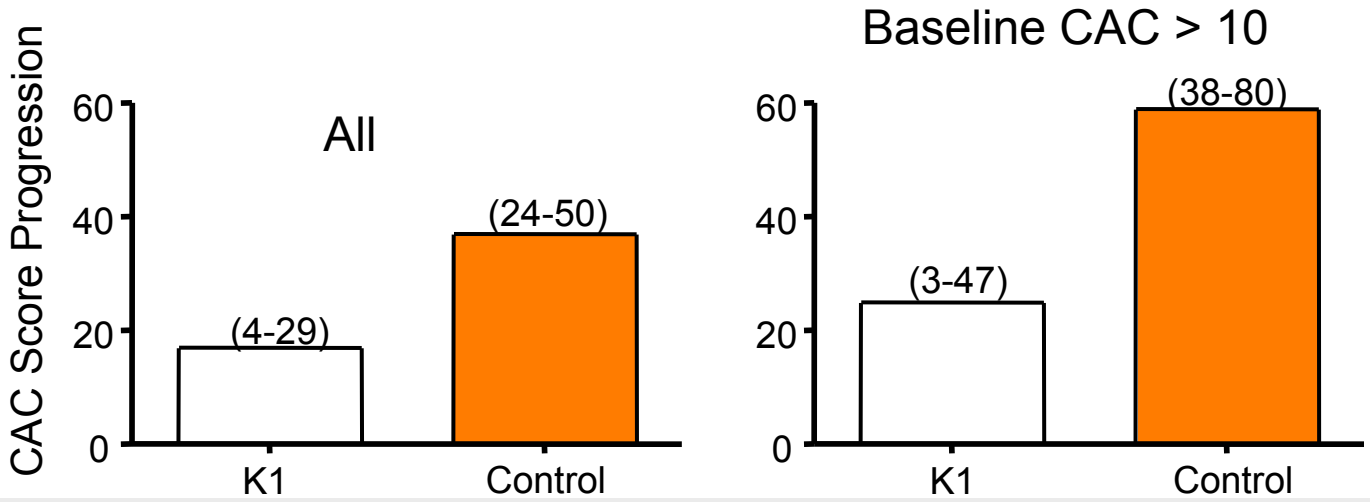
Week 104

- End points: primary = progress of coronary calcification
  secondary = vascular stiffness and biomarkers

Vitamin K supplementation reduces progression of VC

Design: n= 388, mean age 68 years, 500µg K1 supplementation daily
Endpoint: Coronary artery calcification (CAC) progression over 3 y.

Results: Significant differences were only apparent after secondary analysis, restricted to patients >85% adherent to supplementation (n = 367).



K1 supplementation slows the progression of CAC in healthy older adults with preexisting CAC, independent of its effect on total MGP concentrations.
No difference in CV morbidity / mortality between the groups.

Shea et al. AJCN 2009

Take home message

- Vitamin K is effective in activating vitamin K-dependent proteins, such as MGP though current recommended intakes are too low
- Poor vitamin K status → mortality → due to enhanced calcification ?

  (vitamin K-antagonists are a risk factor for calcification)
- New oral anticoagulants (FIIa and FXa inhibitors) provide an alternative in certain patient populations without affecting extra-hepatic vitamin K dependent proteins
- High vitamin K2 (MK7) intake should be considered as a "treatment" option for preventing arterial calcification in both healthy subjects and patients