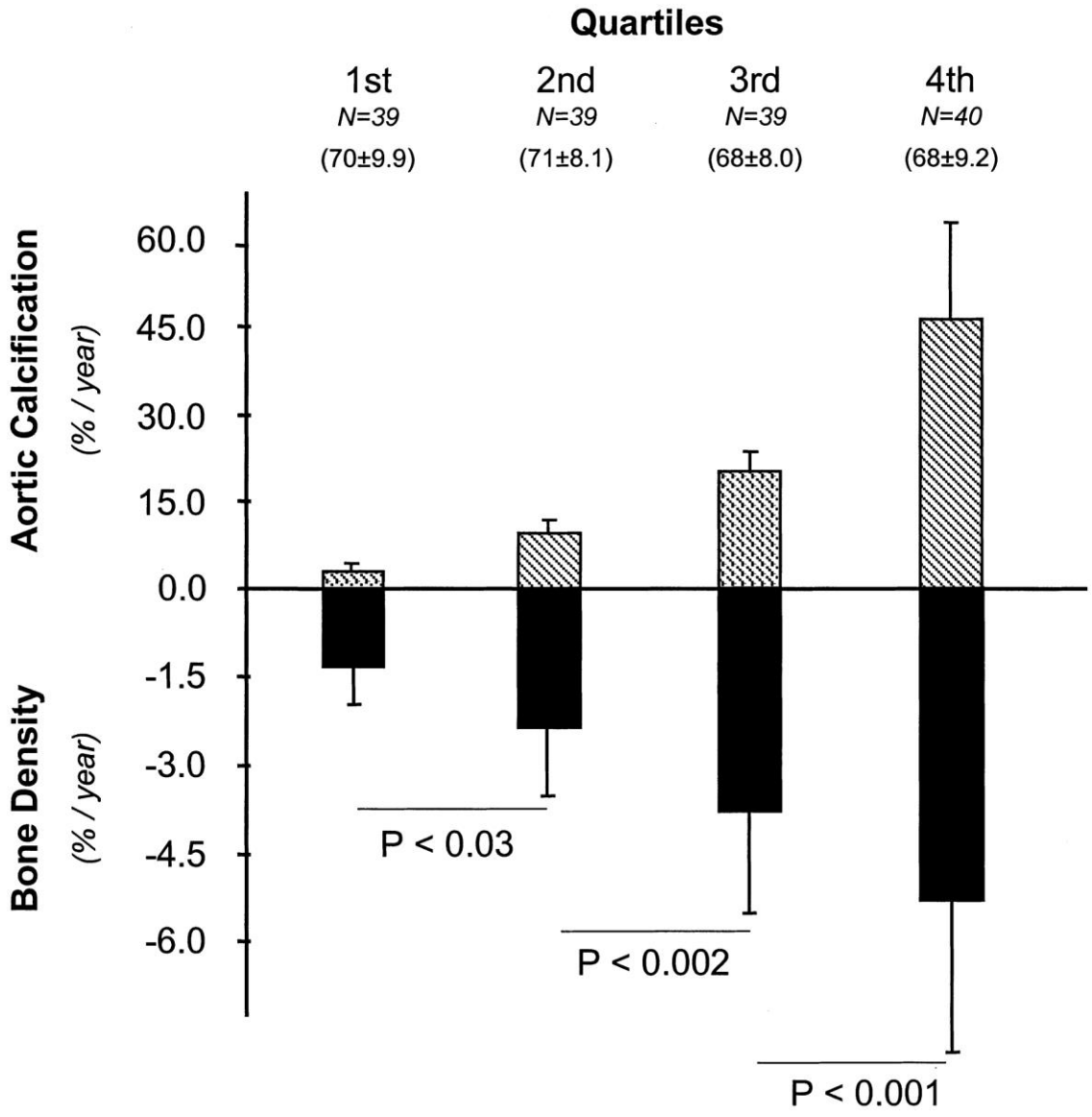
:
Soft Bones – Hard Arteries

The Bone Vascular Axis in CKD/ESRD

Gérard London

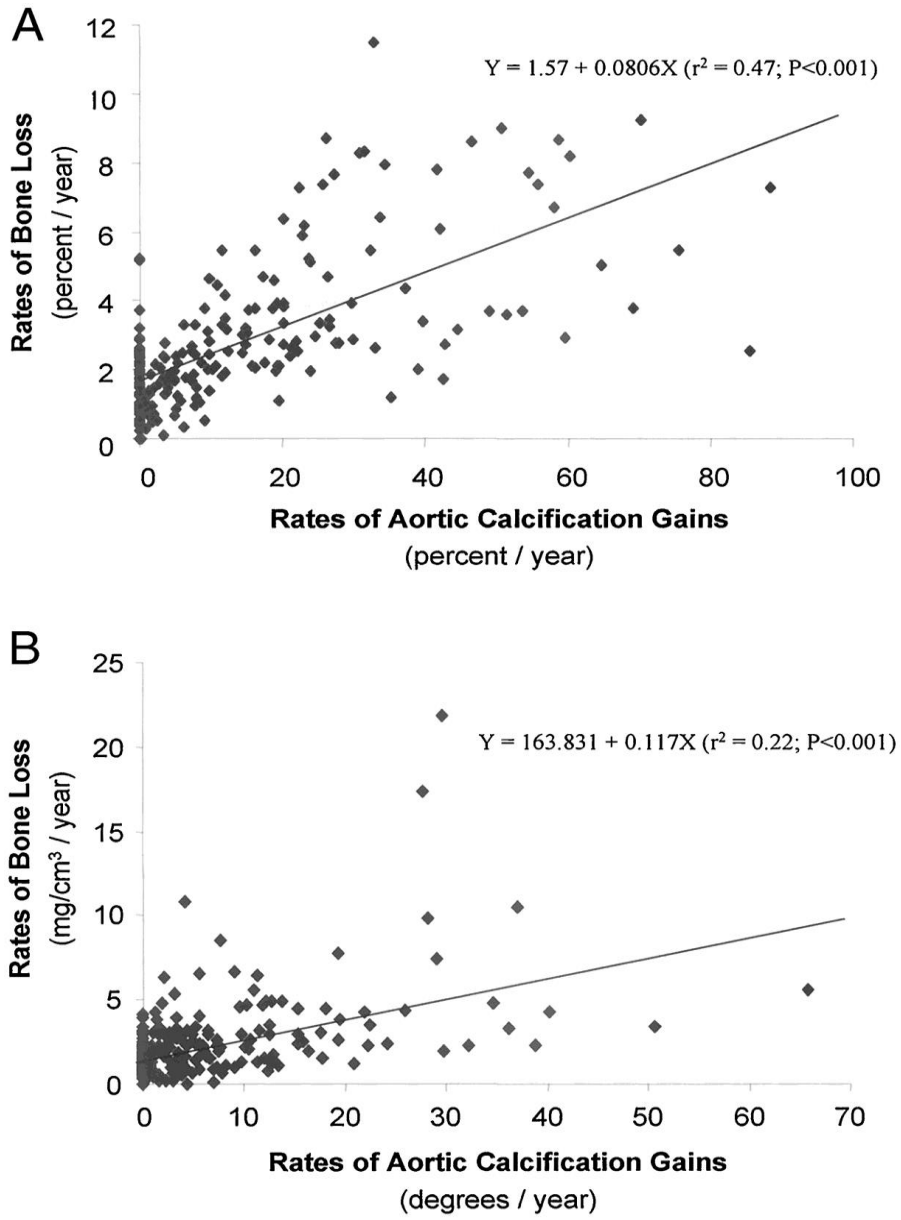
INSERM U970, PARIS

Yearly % gains in aortic calcification and bone loss in the women (n=157) with vascular calcifications at baseline

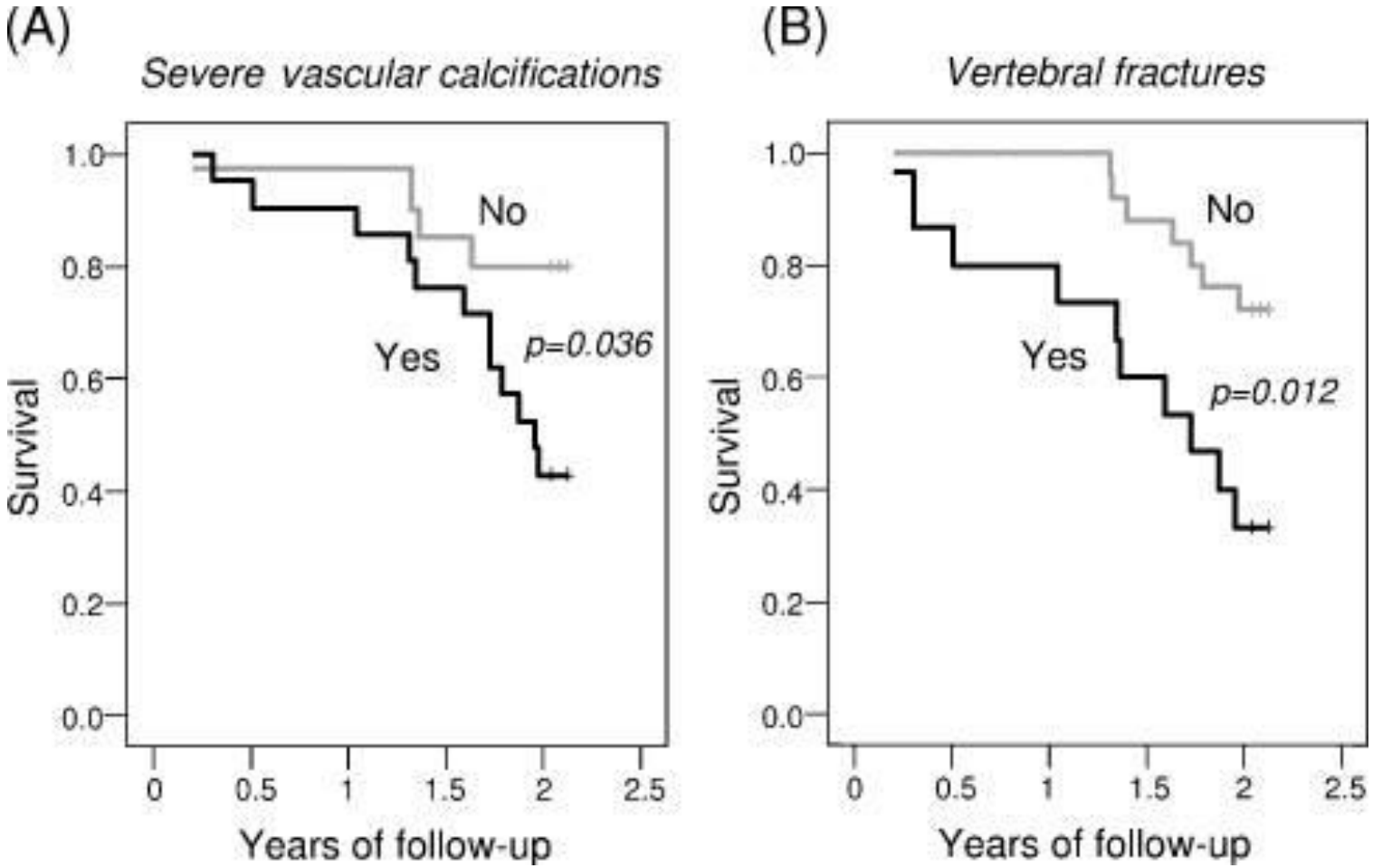


Schulz, E. et al. J Clin Endocrinol Metab 2004;89:4246-4253

Yearly gains in aortic calcification and bone loss as Δ% (A) or Δ in absolute values (B)

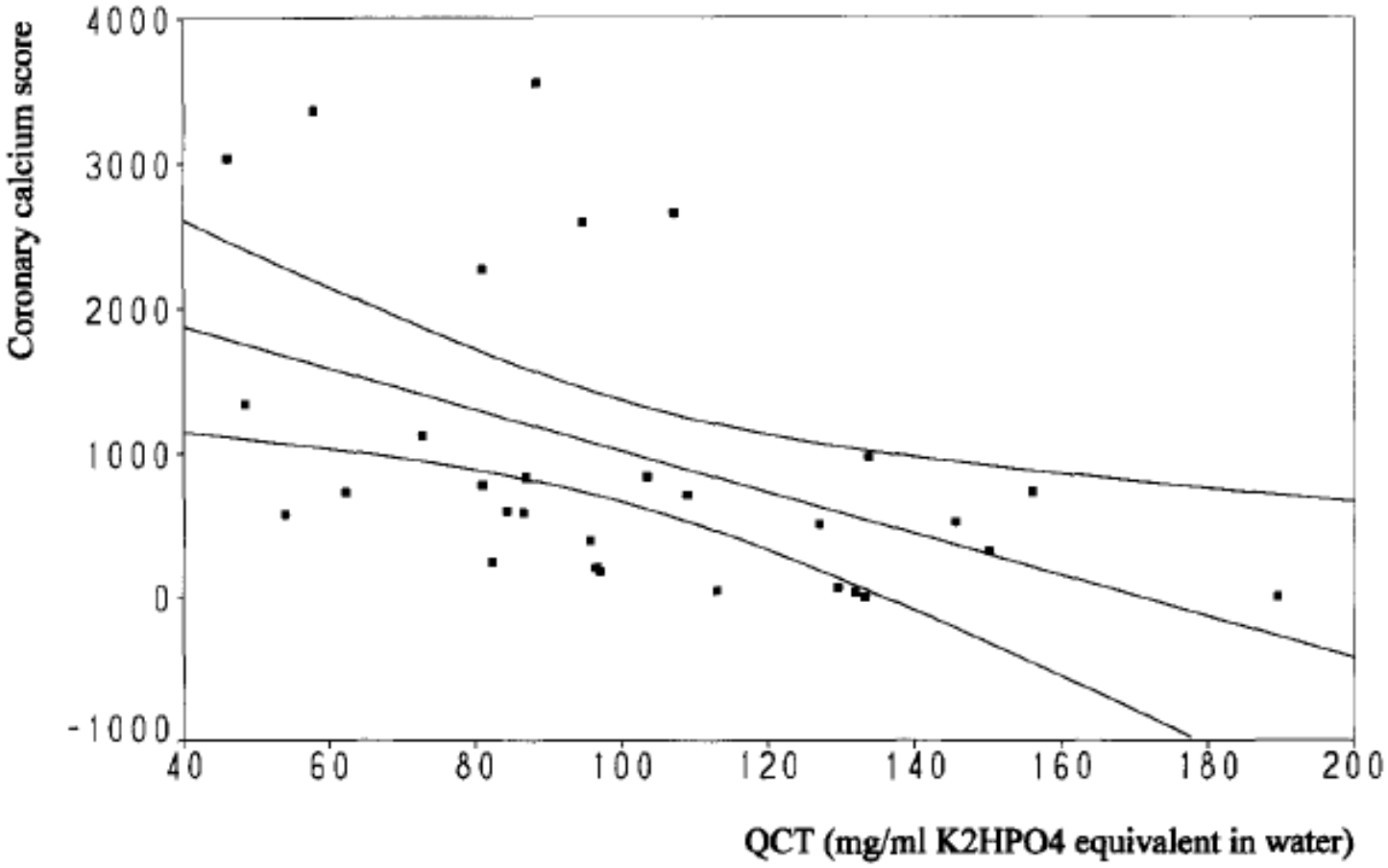


Schulz, E. et al. J Clin Endocrinol Metab 2004;89:4246-4253



Rodriguez –Garcia et al. NDT 2009;24:239

Significant inverse relationship between total coronary artery Calcium score and CT-determined vertebral bone density in hemodialysis patients



Braun J et al. AJKD 1996;27:394

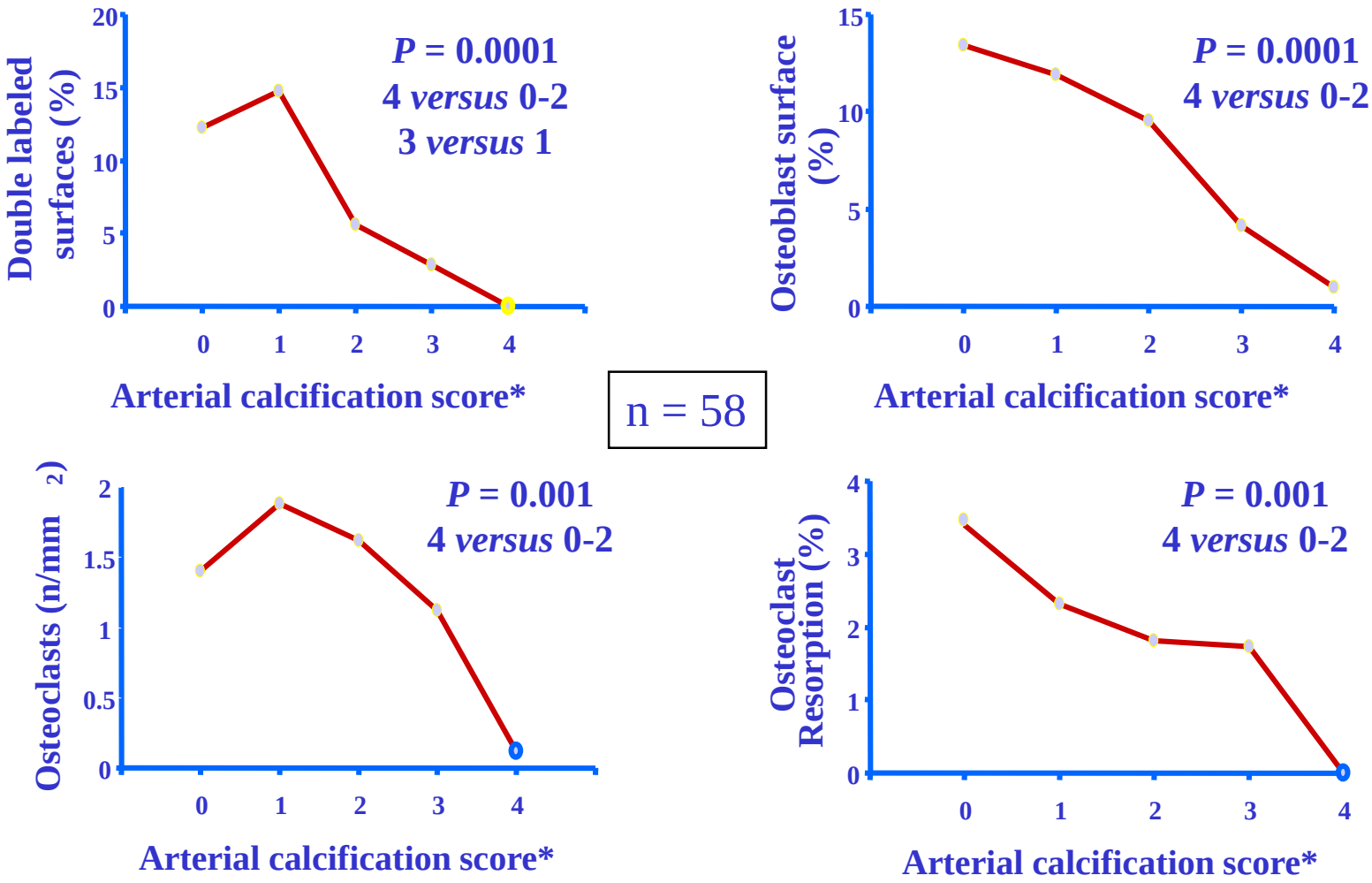
High Prevalence of Vertebral Fractures Assessed by Quantitative Morphometry in Hemodialysis Patients, Strongly Associated with Vascular Calcifications

e

Maria Fusaro, Giovanni Tripepi, Marianna Noale, et al.

Calcified Tissue International, 2013 (ahead of pub.)

Arterial Calcifications and Bone Histomorphometry in ESRD



*Determined by ultrasonography

London GM, *J Am Soc Neph* 15: 1943-1951, 2004

Correlation of bone parameters with coronary artery calcification

	r	p
Osteoid surface	-,209	<0.001
Osteoblast number	-,144	<0.05
Osteoblast surface	-,144	<0.05
Erosion surface	-,150	<0.05
Osteoclast number	-,145	<0.05
Osteoclast surface	-,150	<0.05
Activation frequency	-,217	<0.05

- Several bone parameters showed an inverse correlation with CAC.

Asci G, Ozkahya M, Duman S, Toz H, Savas R, Kayıkcioglu M,
Celik G, Ozbek SS, Basci A, Monier-Faugere MC, Malluche HH, Ok E
On behalf of 'EGE STUDY' and "DIALYSATE CALCIUM STUDY' GROUPS. ERA-EDTA 2006

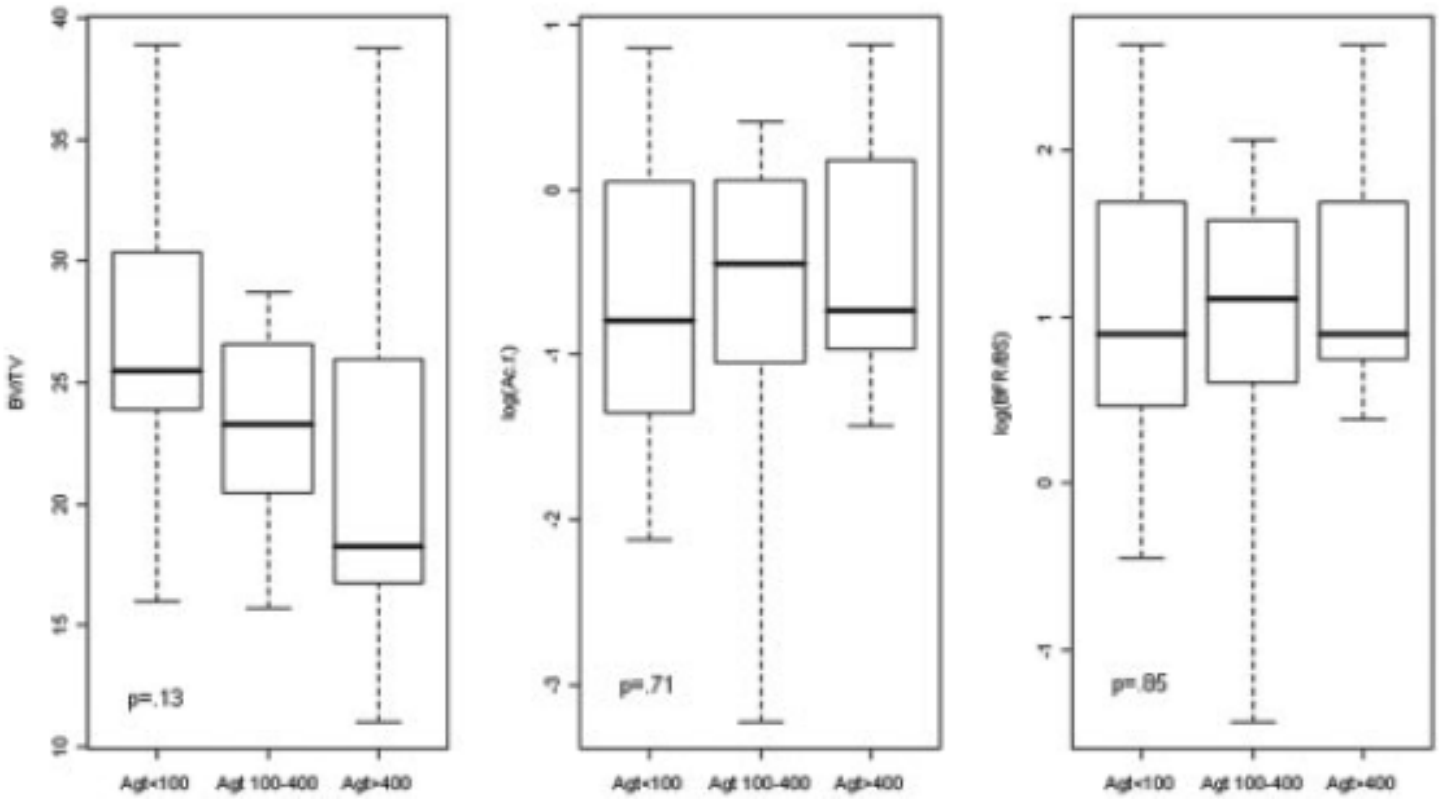
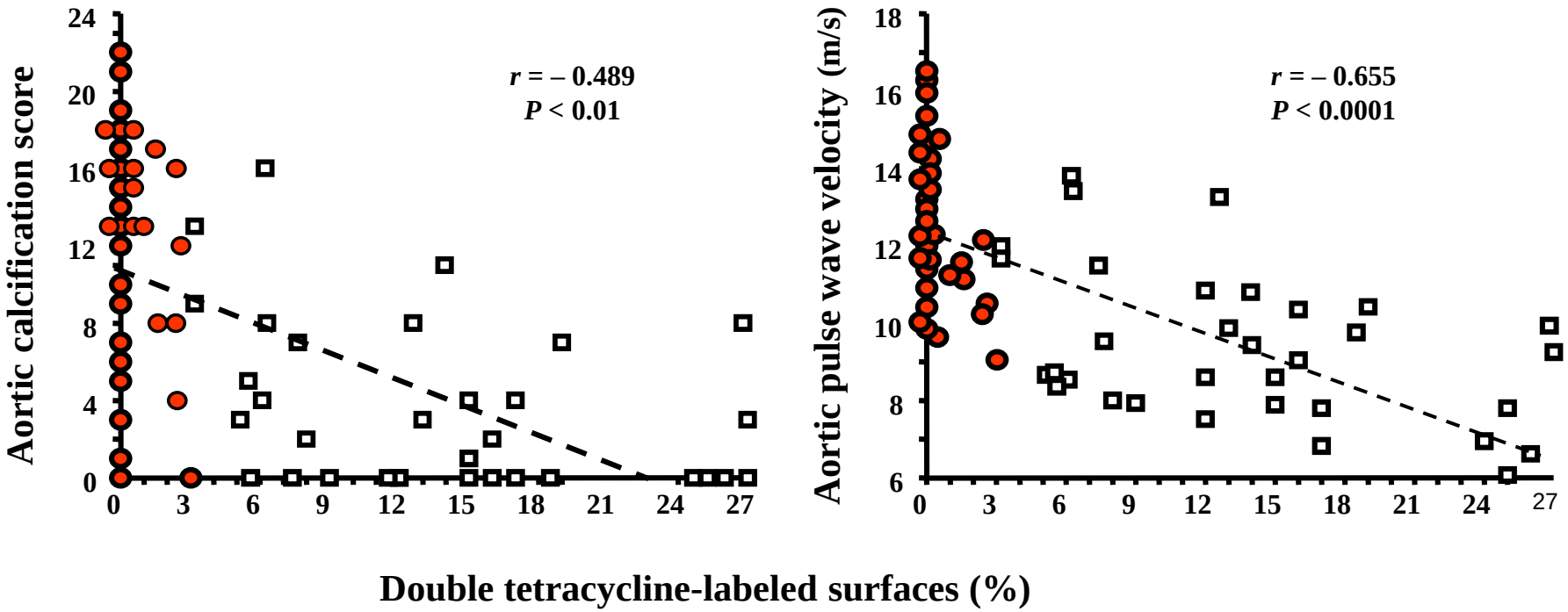


Figure 1. Distribution of values for bone volume/tissue volume (BV/TV), activation frequency (Ac.f.), and bone formation rate/bone surface (BFR/BS) among the Agatston (Agt.) score classes <100, 100 to 400, and >400. Box = median, 25 to 75%; T-bars = minimum and maximum values.

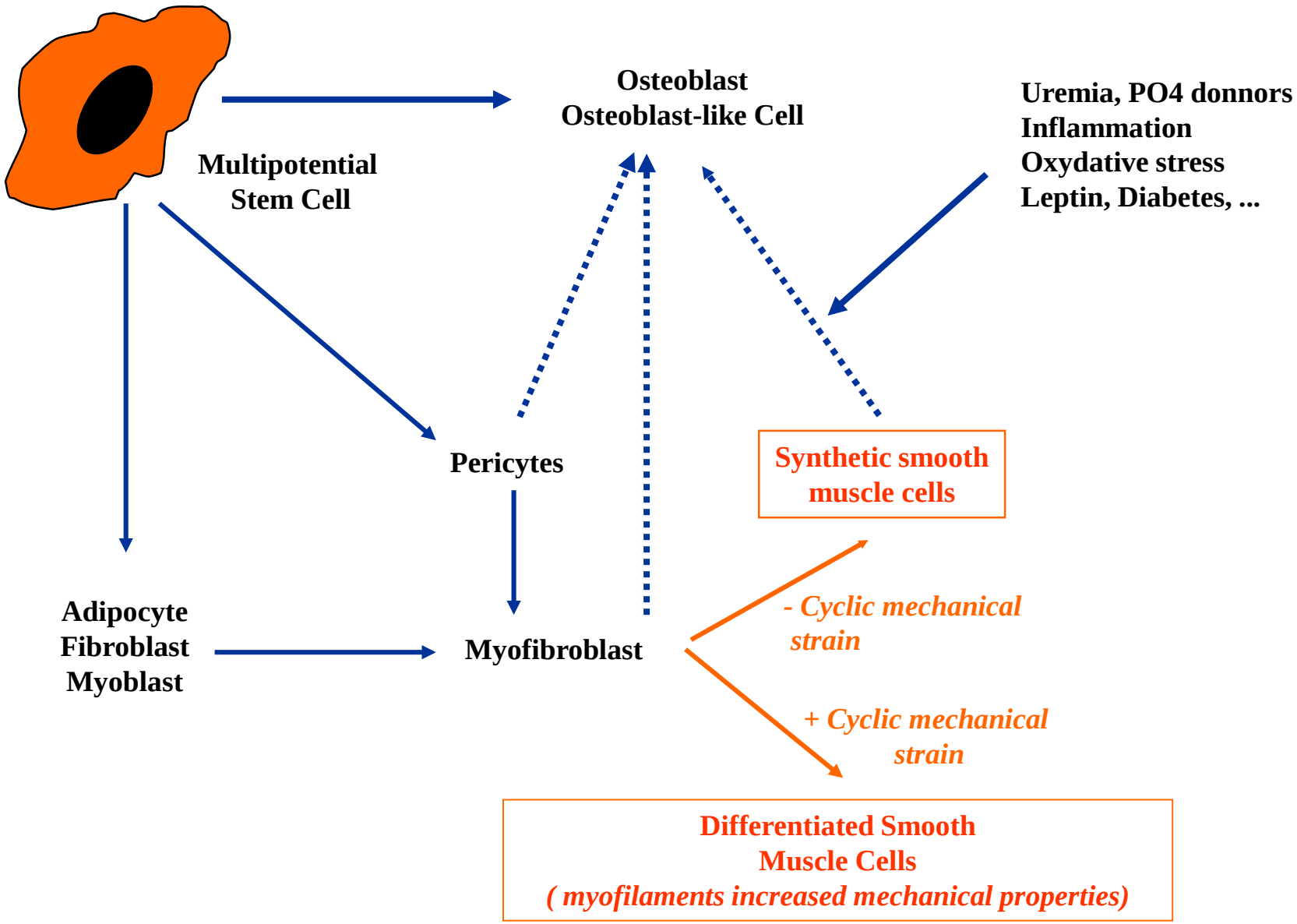
Adragao et al CJANS 2009;4:450



London GM et al JASN 2008

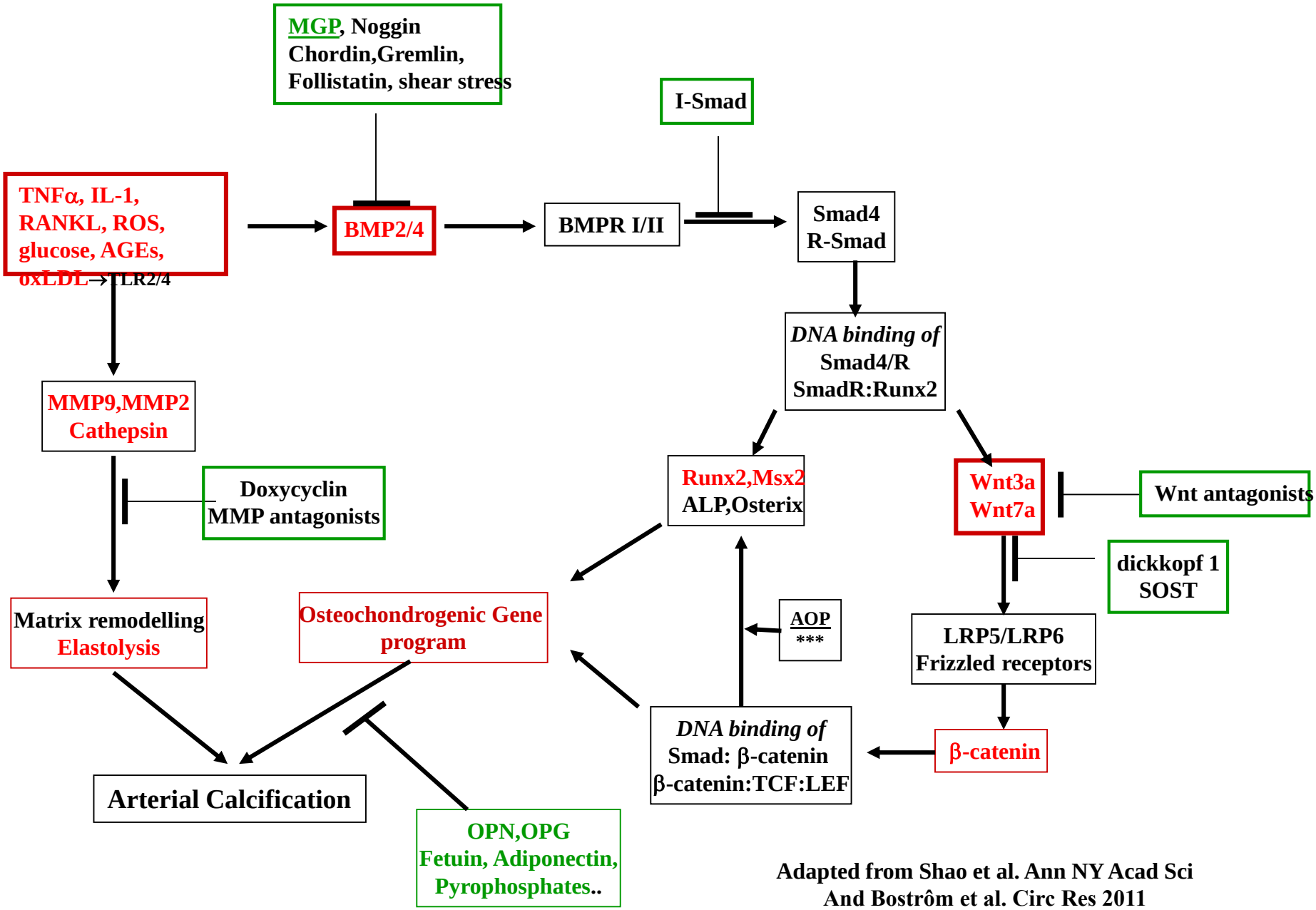
Possible Mechanisms Associating Bone and Arterial Disorders

- Common factors: inflammation, tobacco, lipid disorders, estrogen deficiency, aging, diabetes, oxidative stress, …
- Generalized arterial disease involving bone arteries – reduced circulation to bone
- Primary bone alteration affecting systemic arteries


Osteoblast
Osteoblast-like Cell
Uremia, PO4 donnors
Inflammation
Oxydative stress
Leptin, Diabetes, ...
Multipotential
Stem Cell
Pericytes
Synthetic smooth
muscle cells
Adipocyte
Fibroblast
Myoblast
Myofibroblast
- Cyclic mechanical
strain
+ Cyclic mechanical
strain
Differentiated Smooth
Muscle Cells
(myofilaments increased mechanical properties)

BMP- Msx2-Wnt signaling in arterial calcifications

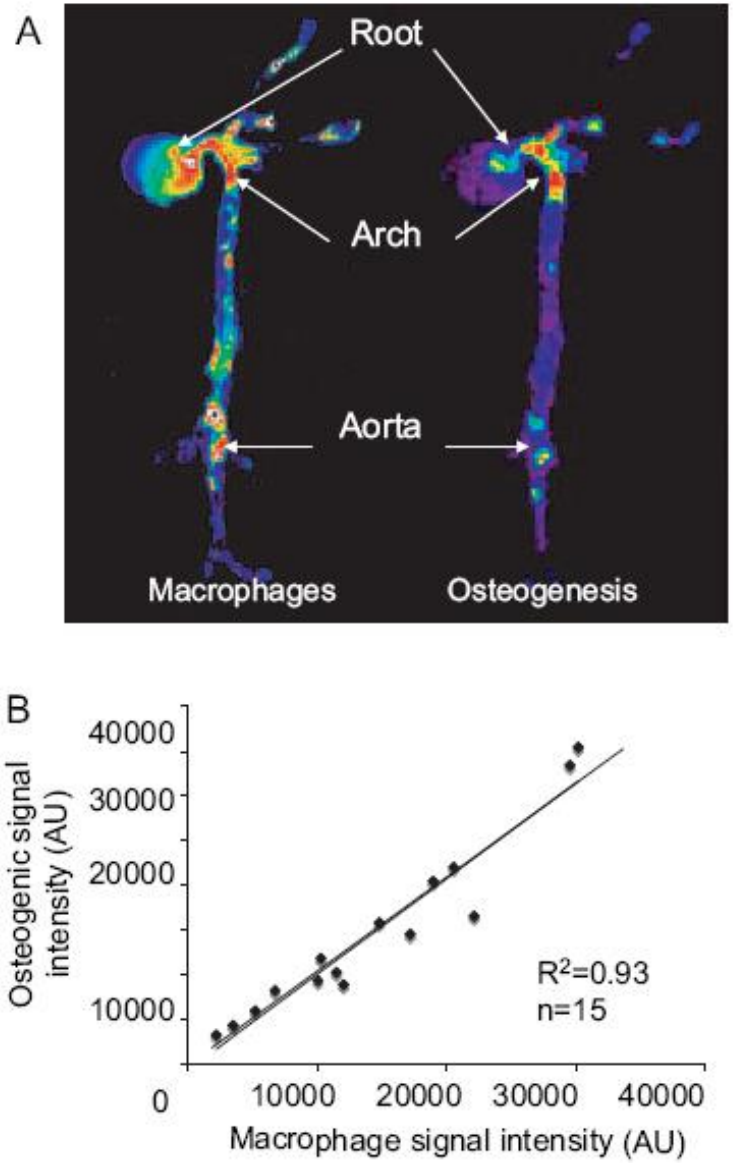
AOP *** (Accentuated Osteogenic Program)



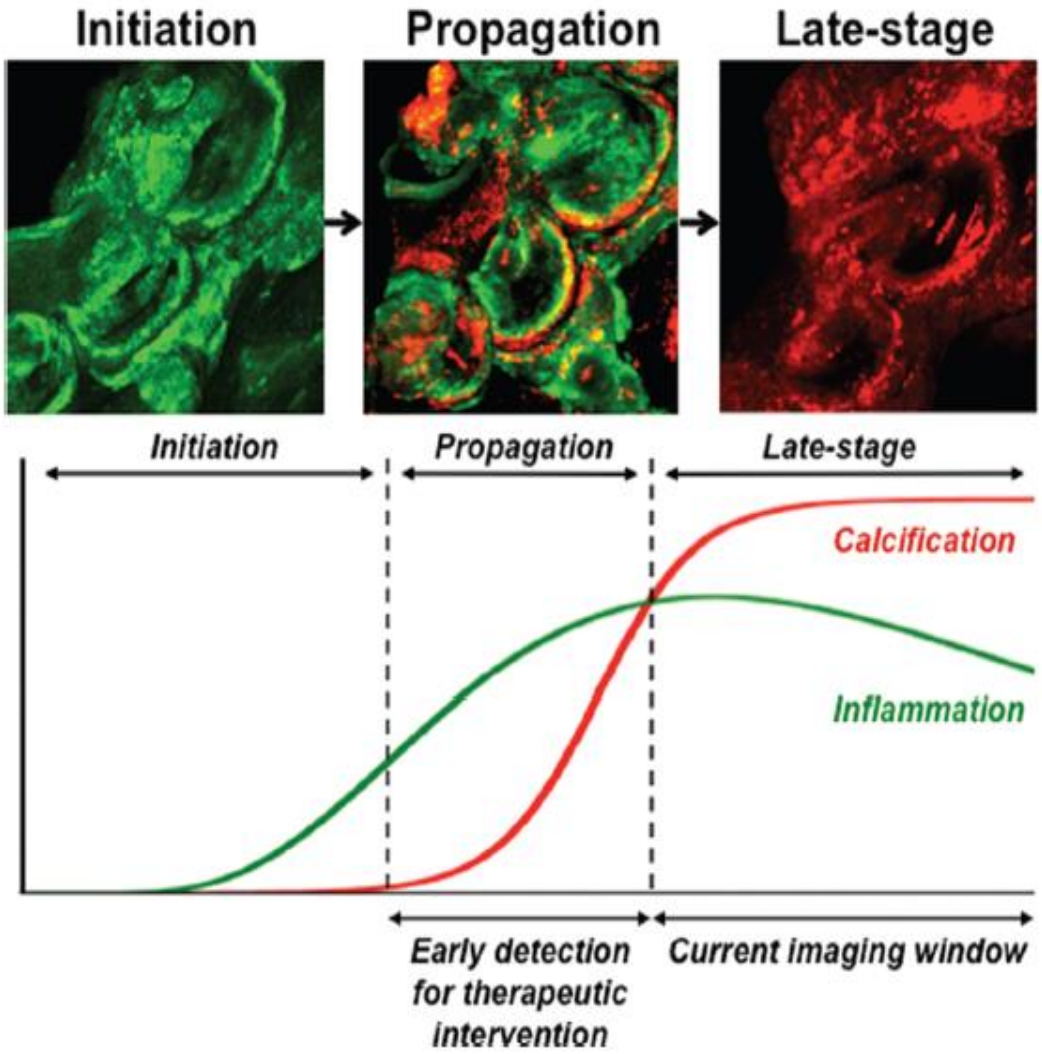
Adapted from Shao et al. Ann NY Acad Sci
And Bostrôm et al. Circ Res 2011

Molecular imaging of atherosclerotic calcifications

Simultaneous mapping correlated macrophage burden and osteogenic activity

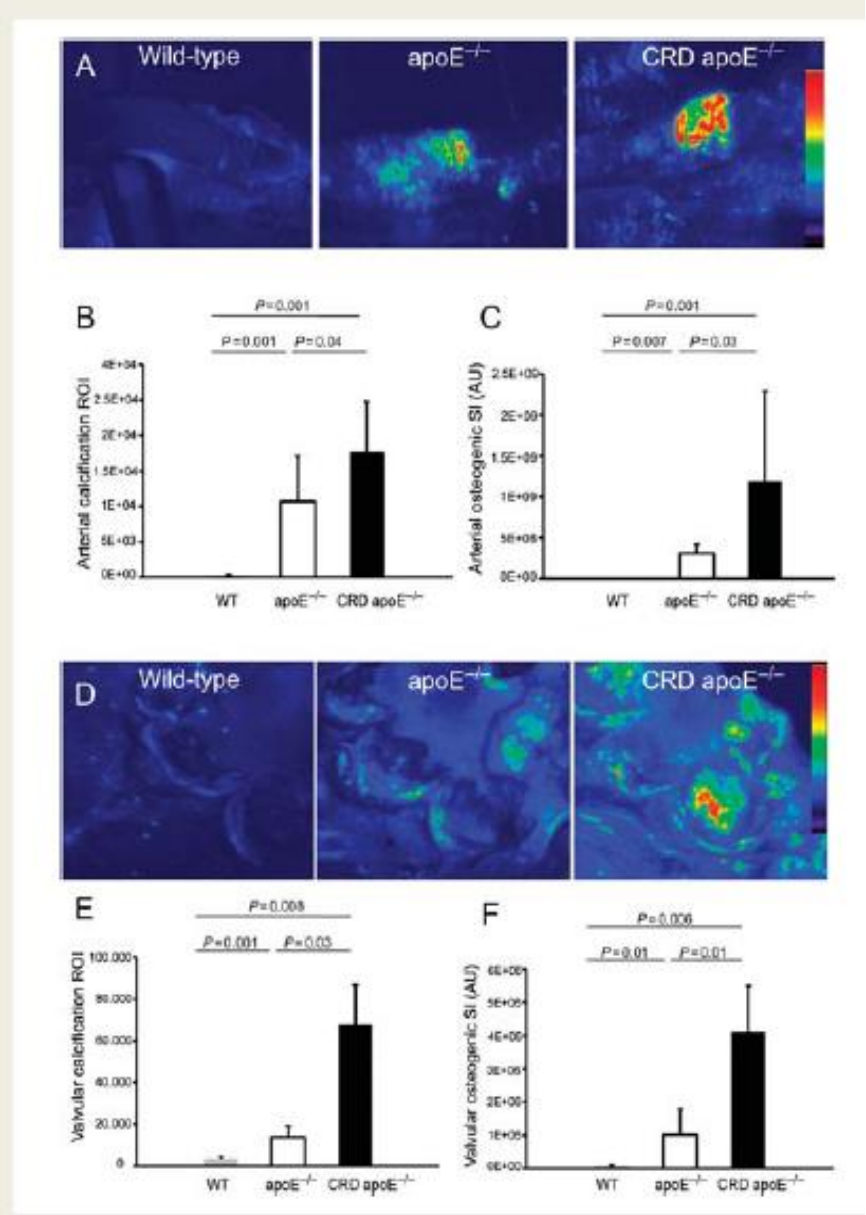


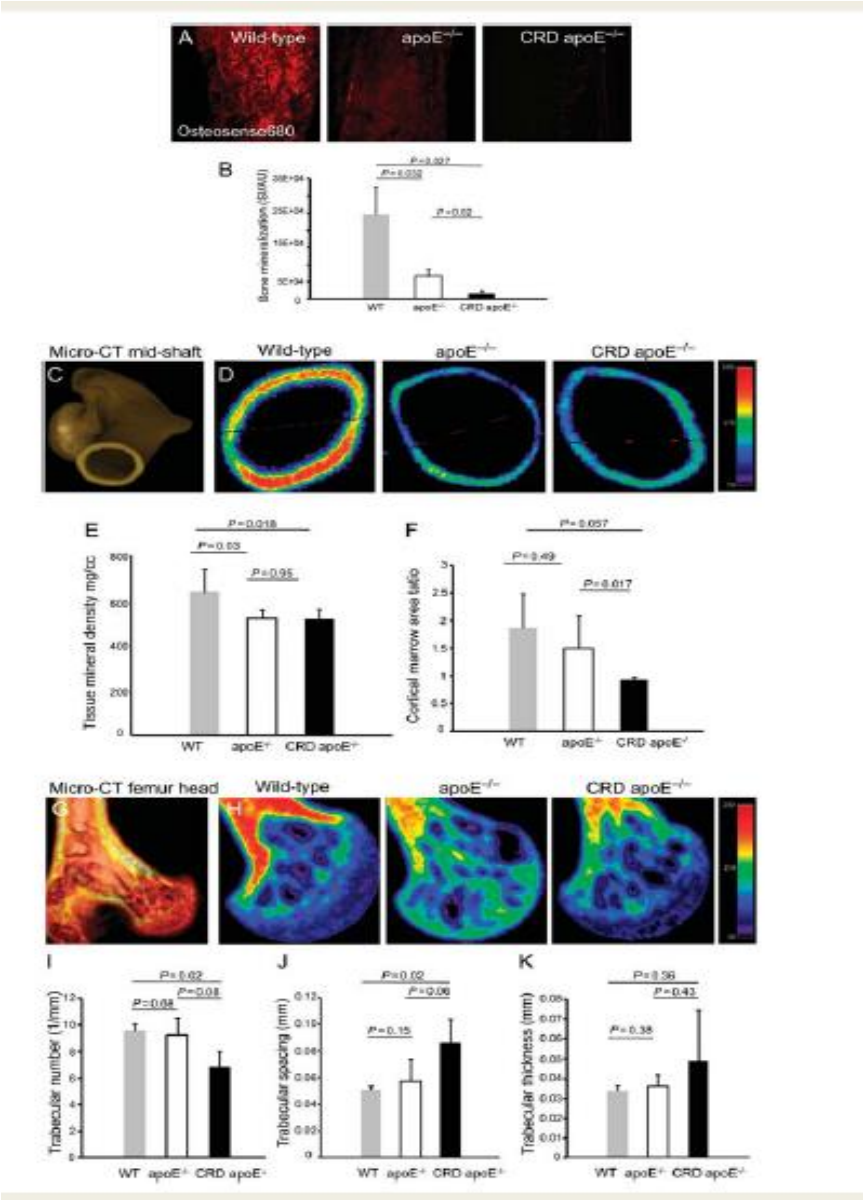
Aikawa et al. Circulation 2007;116:2841-50



Aikawa E Circ Res 2011

Induction of osteogenesis in carotid arteries and aortic valves of apoE−/− and CKD apoE−/− mice, and loss of bone mineral density and alterations in microarchitecture in femurs

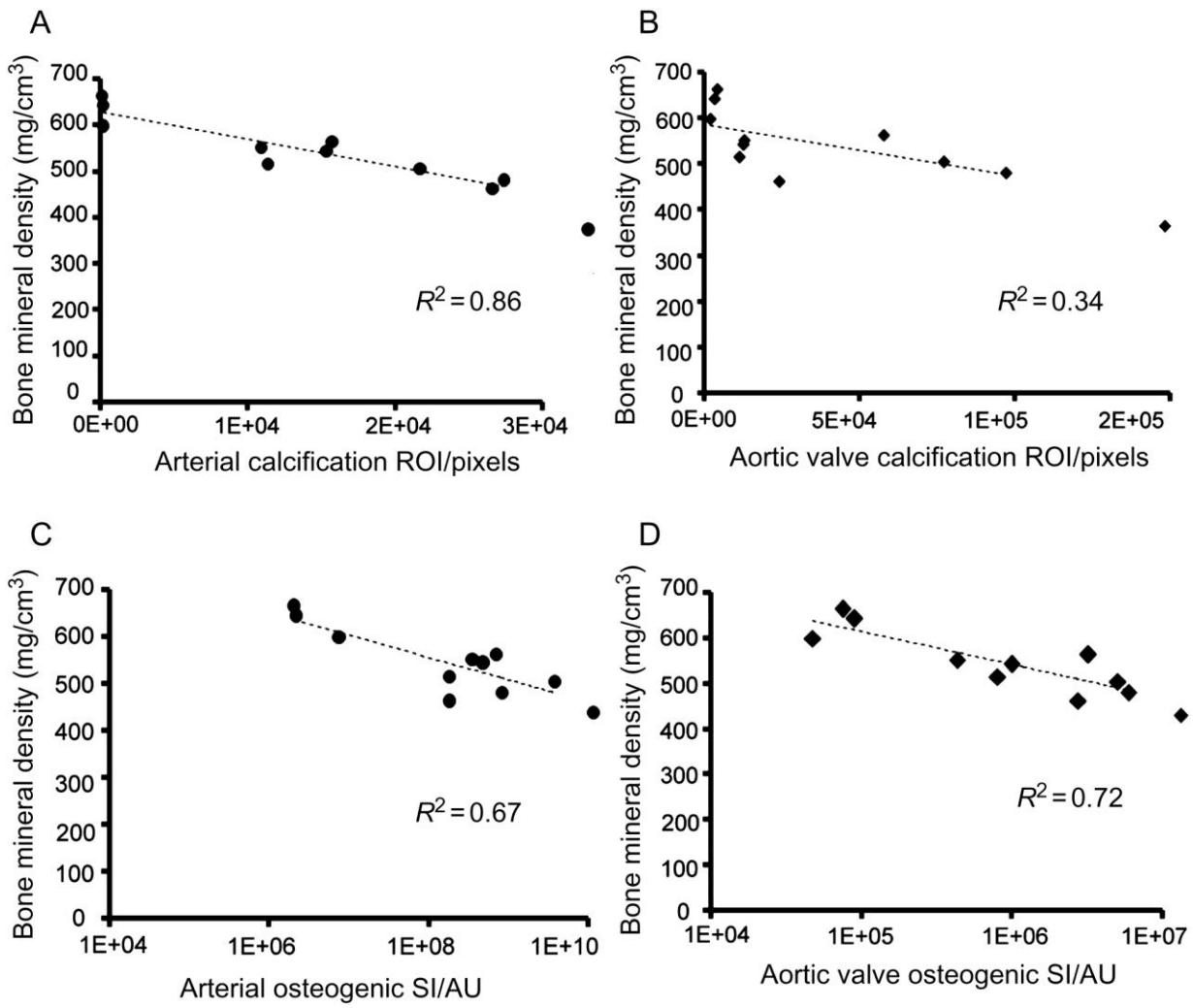




Hjortnaes J et al. Eur Heart J 2010;31:1975-1984

Osteosense-calcification intravital marker

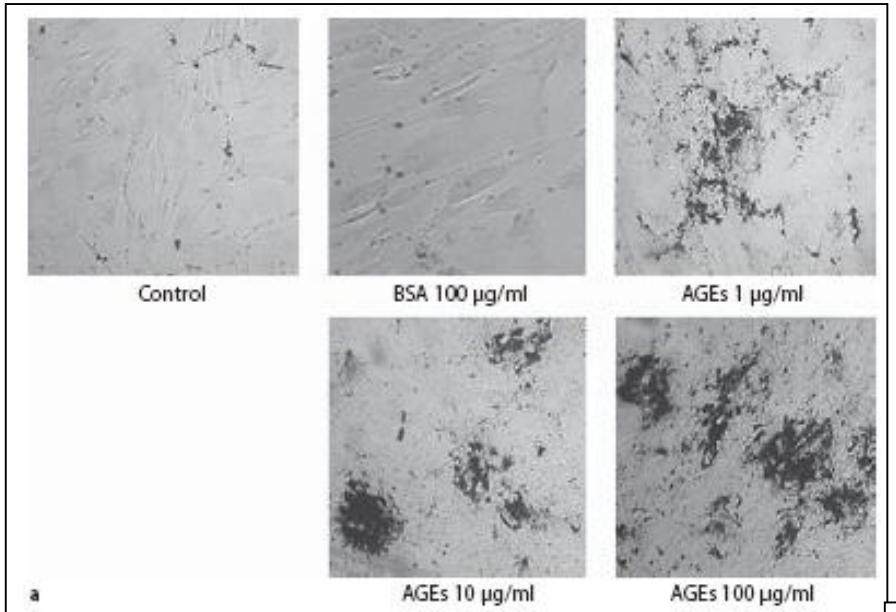
Inverse correlation of bone tissue mineral density with arterial and aortic valve calcification.

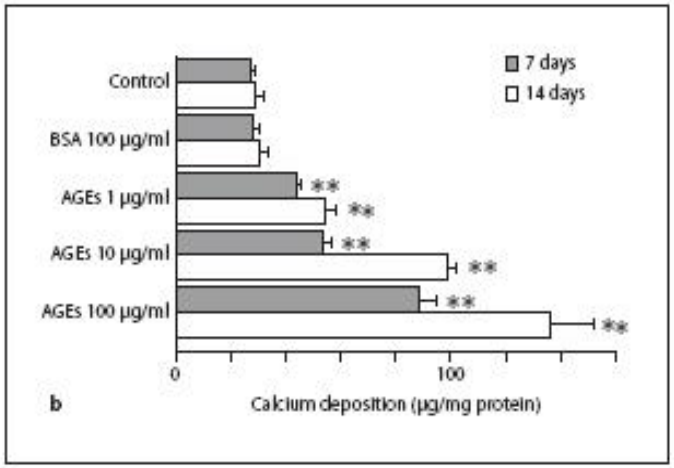


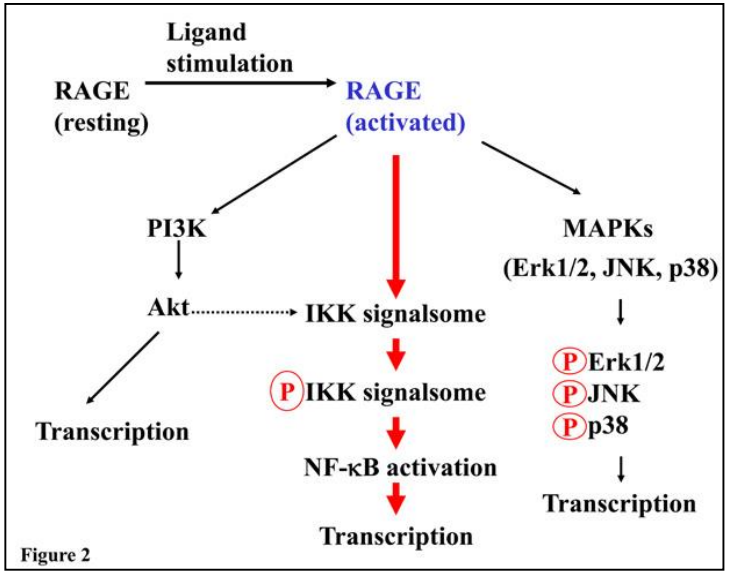
Hjortnaes J et al. Eur Heart J 2010;31:1975-1984

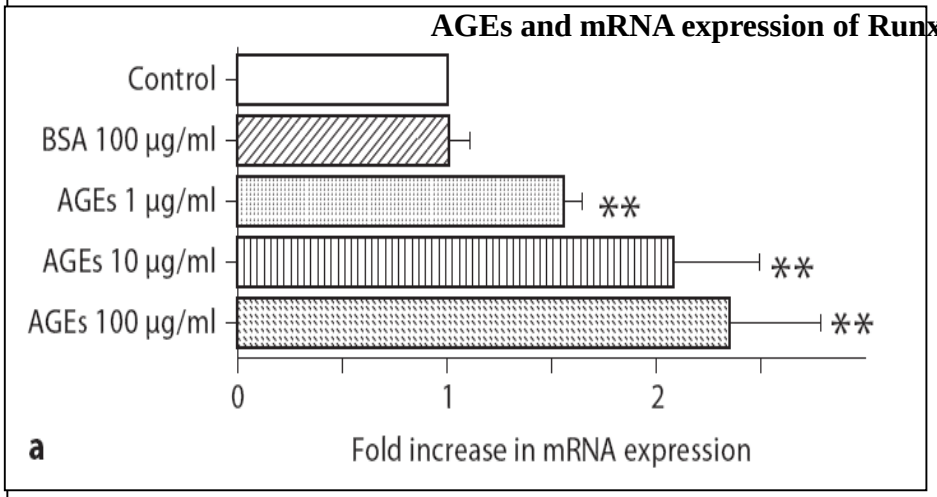
Advanced glycation end products induce calcification of VSMC through RAGE/p38MAPK and upregulation of Runx2

Tanikawa T et al.J Vasc Res 2009;46:572-580









Bone. 2007 February ; 40(2): 345–353.

Advanced Glycation Endproducts Stimulate Osteoblast Apoptosis Via the MAP Kinase and Cytosolic Apoptotic Pathways

Mani Alikhani[^], **Zoubin Alikhani**[^], **Coy Boyd**[^], **Christine M. MacLellan**[^], **Markos Raptis**[^], **Rongkun Liu**[^], **Nicole Pischon**[^], **Philip C. Trackman**[^], **Louis Gerstenfeld**[*], and **Dana T. Graves**[^]

We have previously shown that diabetes significantly enhances apoptosis of osteoblastic cells *in vivo* and that the enhanced apoptosis contributes to diabetes impaired new bone formation. A potential mechanism is enhanced apoptosis stimulated by advanced glycation endproducts (AGEs). To investigate this further, an advanced glycation product, carboxymethyl lysine modified collagen (CML-collagen) was injected *in vivo* and stimulated a 5 fold increase in calvarial periosteal cell apoptosis compared to unmodified collagen. It also induced apoptosis in primary cultures of human or neonatal rat osteoblastic cells or MC-3T3-E1 cells *in vitro*. Moreover, the apoptotic effect was largely mediated through RAGE receptor. CML-collagen increased p38 and JNK activity 3.2 and 4.4 fold, respectively. Inhibition of p38 and JNK reduced CML-collagen stimulated apoptosis by 45% and 59% and by 90% when used together ($P<0.05$). The predominant apoptotic pathway induced by CML-collagen involved caspase-8 activation of caspase-3 and was independent of NF-κB activation. When osteoblastic cells were exposed to a long-term low dose incubation with CML-collagen there was a higher degree of apoptosis compared to short term incubation. In more differentiated osteoblastic cultures apoptosis was enhanced even further. These results indicate that advanced glycation endproducts, which accumulate in diabetic and aged individuals may promote apoptosis of osteoblastic cells and contribute to deficient bone formation.

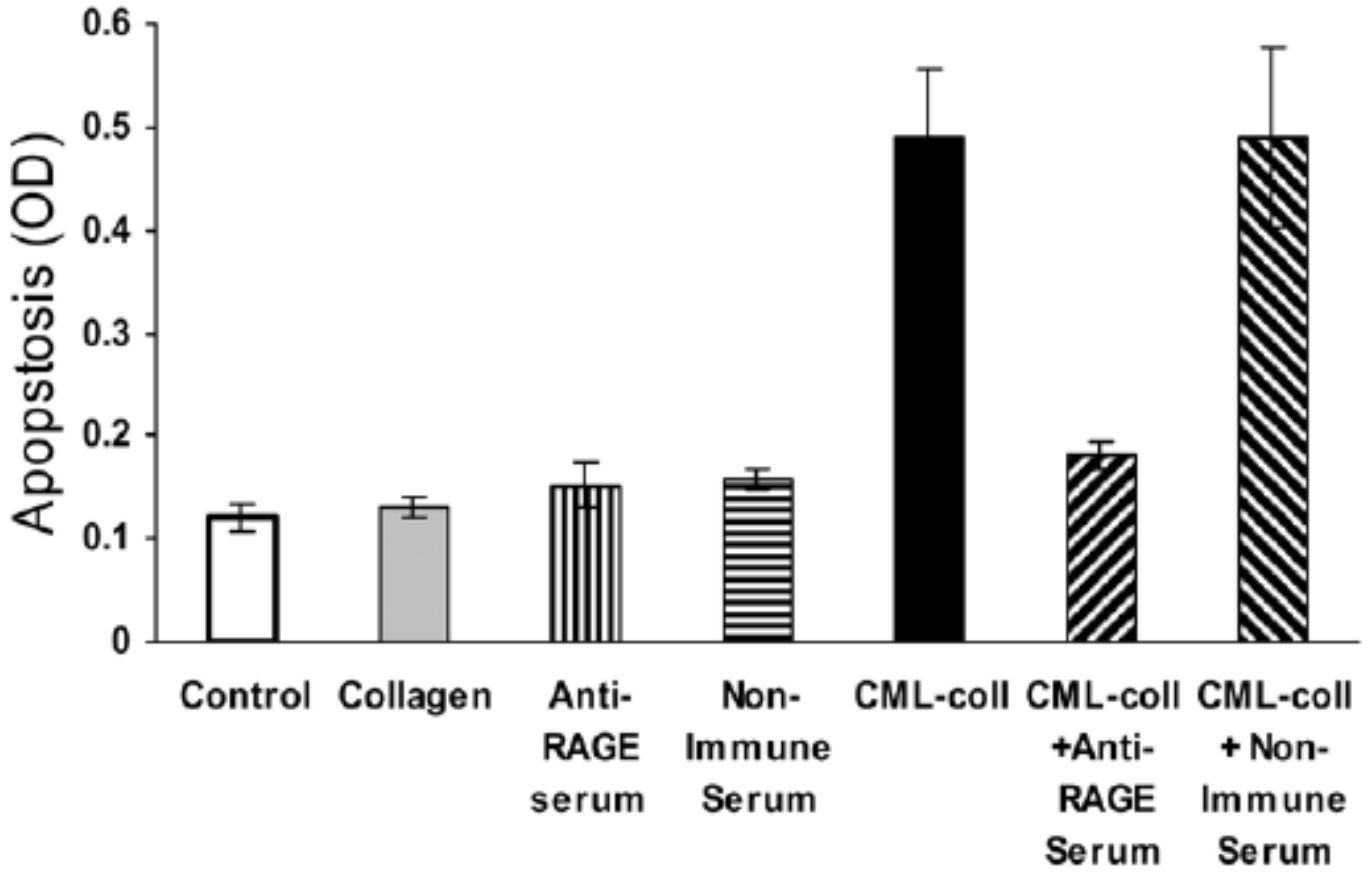


Figure 3. CML-collagen inducedCollagen stimulates apoptosis is mediated through RAGE

CML-coll: Carboxymethyl lysine modified collagen

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 8, pp. 5691–5703, February 23, 2007

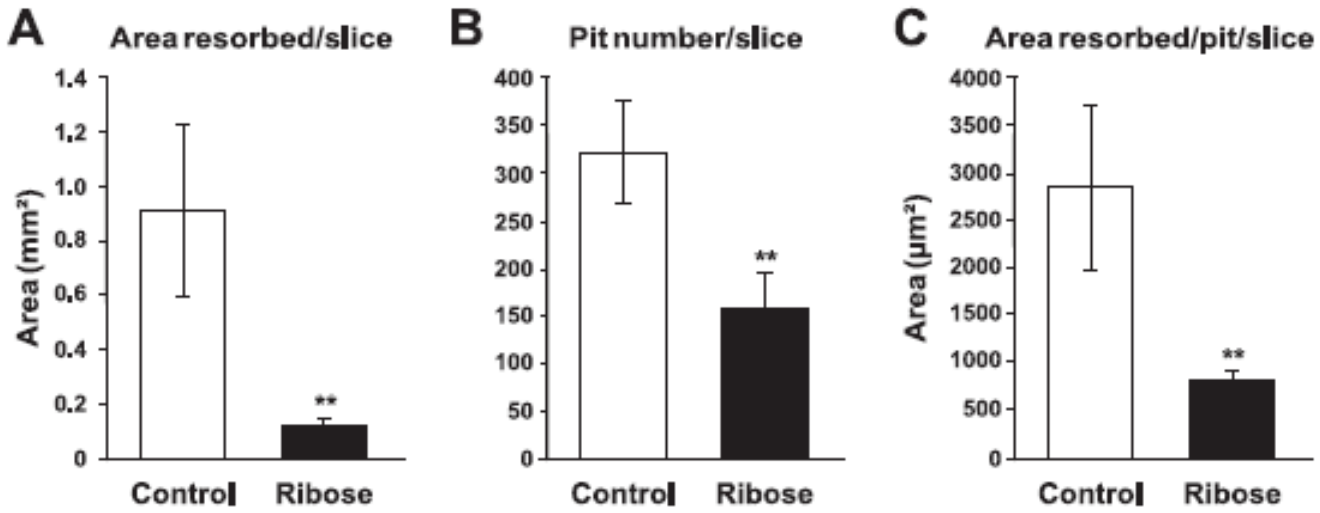
Non-enzymatic Glycation of Bone Collagen Modifies Osteoclastic Activity and Differentiation*[S]

Received for publication, November 13, 2006, and in revised form, November 30, 2006 Published, JBC Papers in Press, December 1, 2006, DOI 10.1074/jbc.M610536200

Ulrich Valcourt[‡1,2], Blandine Merle[‡1], Evelyne Gineyts[‡], Stéphanie Viguet-Carrin[‡], Pierre D. Delmas[‡], and Patrick Garnero[§]

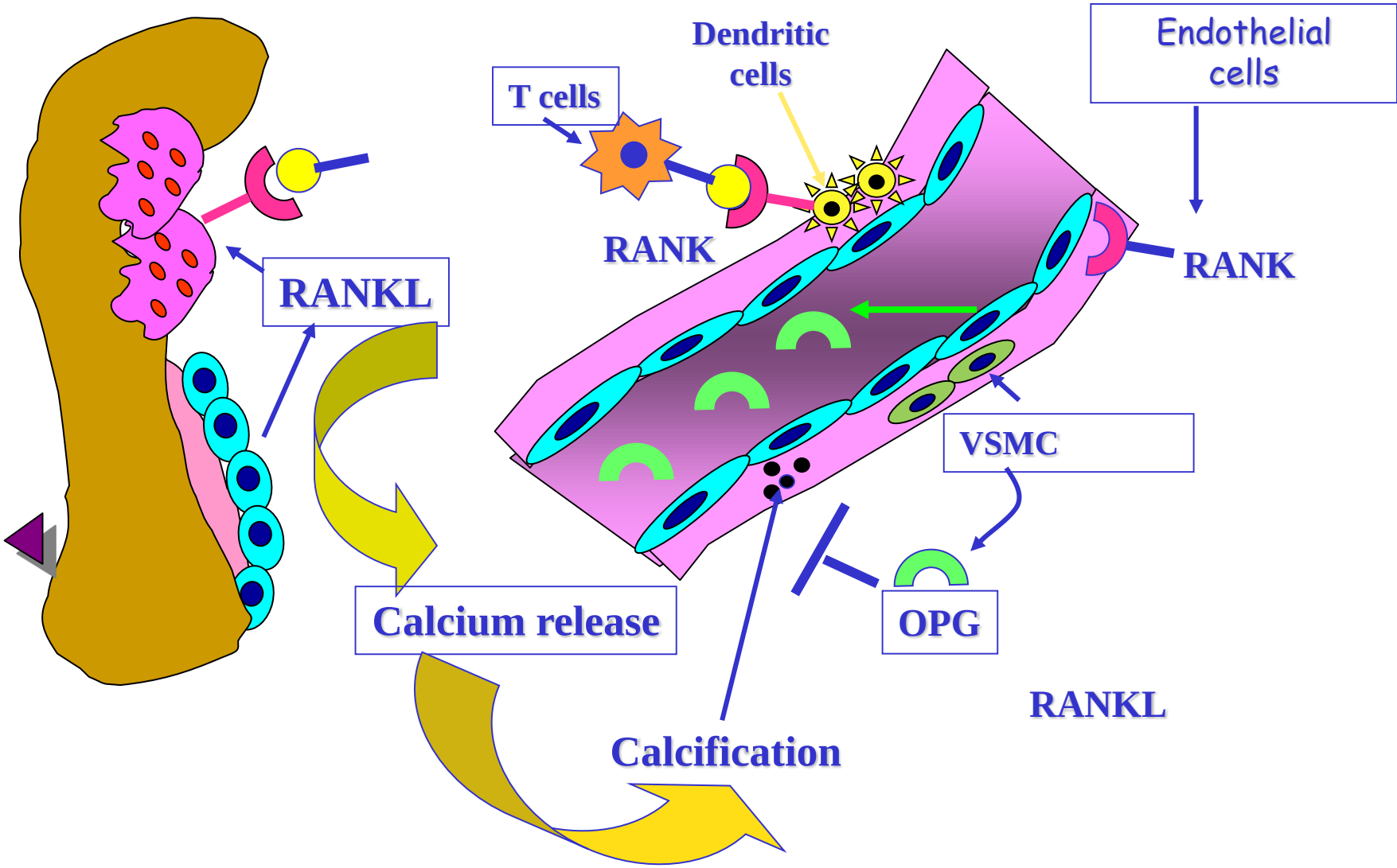
that the resorption process was markedly inhibited when mature osteoclasts were seeded on slices containing matrix pentosidine, a well characterized AGE. More specifically, the total area resorbed per slice, and the area degraded per resorption lacuna created by osteoclasts, were significantly decreased in AGE-containing slices. This inhibition of bone resorption was confirmed by a marked reduction of the release of type I collagen fragments generated by the collagenolytic enzymes secreted by osteoclasts in the culture medium of AGE-modified mineralized matrices. This effect is likely to result from decreased solubility of collagen molecules in the presence of AGEs, as documented by the reduction of pepsin-mediated digestion of AGE-containing collagen. We found that AGE-modified BSA totally inhibited osteoclastogenesis *in vitro*, most likely by impairing the commitment of osteoclast progenitors into pre-osteoclastic cells. Although the mechanisms remain unknown, AGEs might interfere with osteoclastic differentiation and activity through their interaction with specific cell-surface receptors, because we showed that both osteoclast progenitors and mature osteoclasts expressed different AGEs receptors, including receptor for AGEs (RAGEs). These results suggest that AGEs decreased osteoclast-induced bone resorption, by altering not only the structural integrity of bone matrix proteins but also the osteoclastic differentiation process. We suggest that AGEs may play a role in the alterations of bone remodeling associated with aging and diabetes.

AGEs Modify Osteoclastic Activity and Differentiation



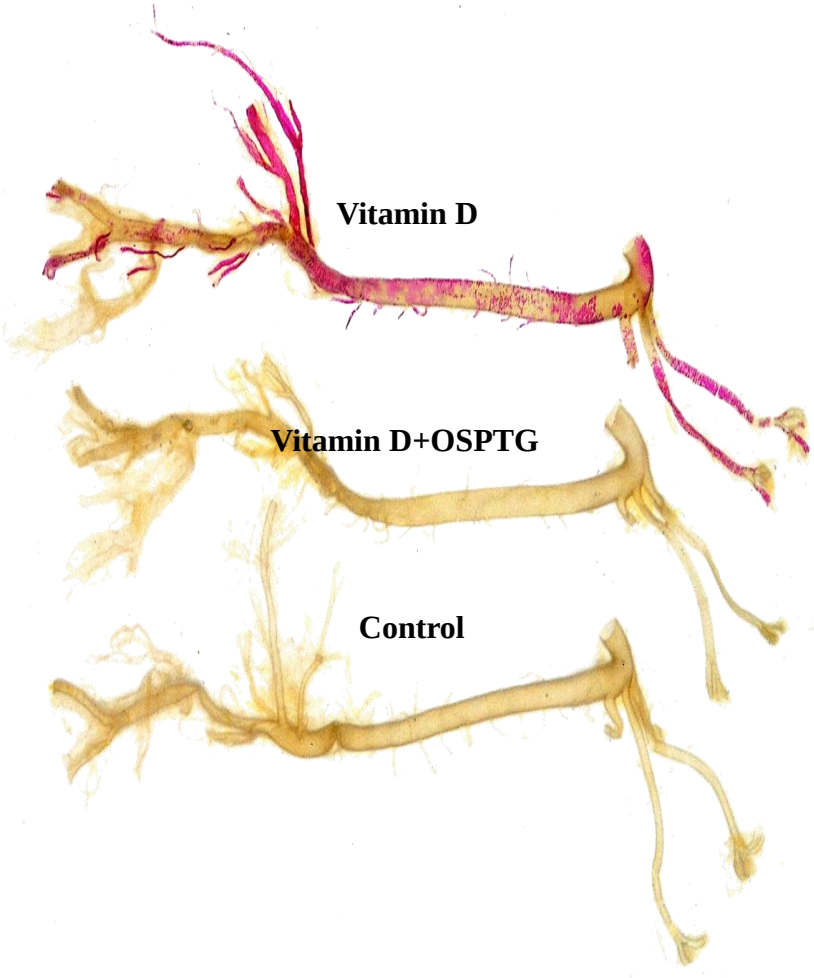
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 8, pp. 5691–5703,

Hypothetical role of RANKL and OPG in the cross-talk between the skeletal and vascular systems



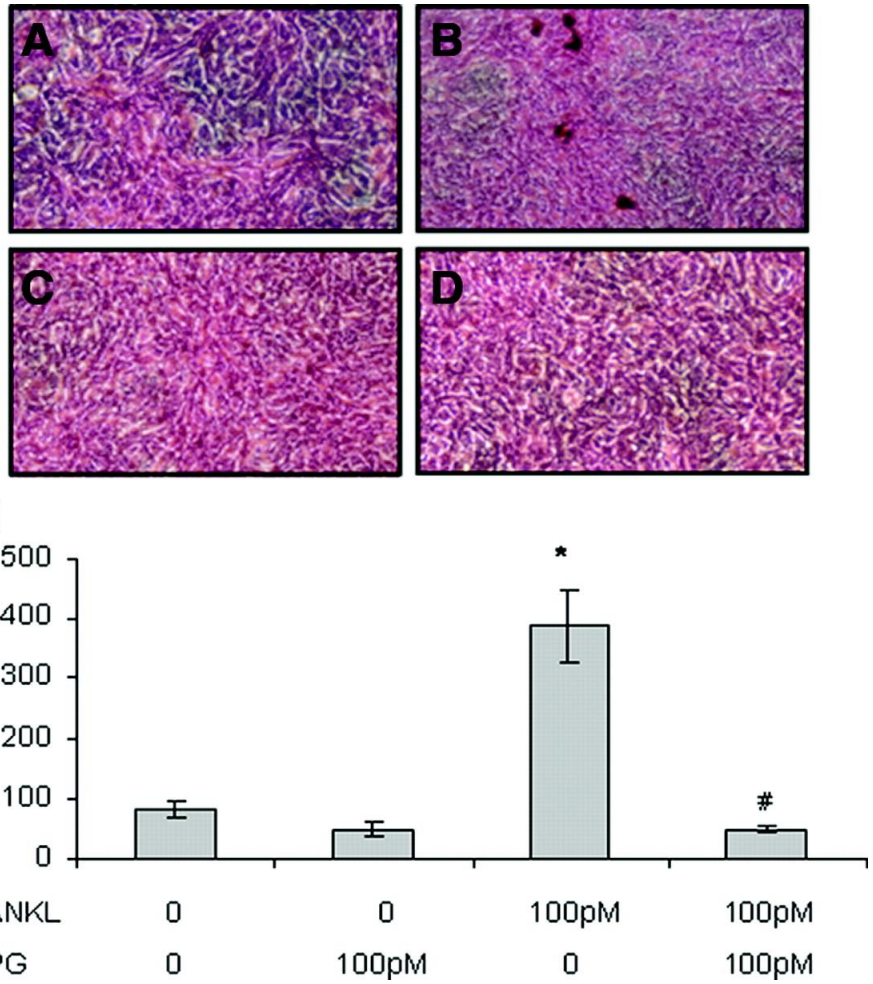
Schoppet et al; Arterioscler Thromb Vasc Biol, 22: 2002.549

Effect of Osteoprotegerin on Vit-D induced calcifications



Price PA et al Arterioscl Thromb Vasc Biol 2001;21:1610

Effect of the incubation of rat VSMCs with RANKL (100 pmol/L) and OPG (100 pmol/L)



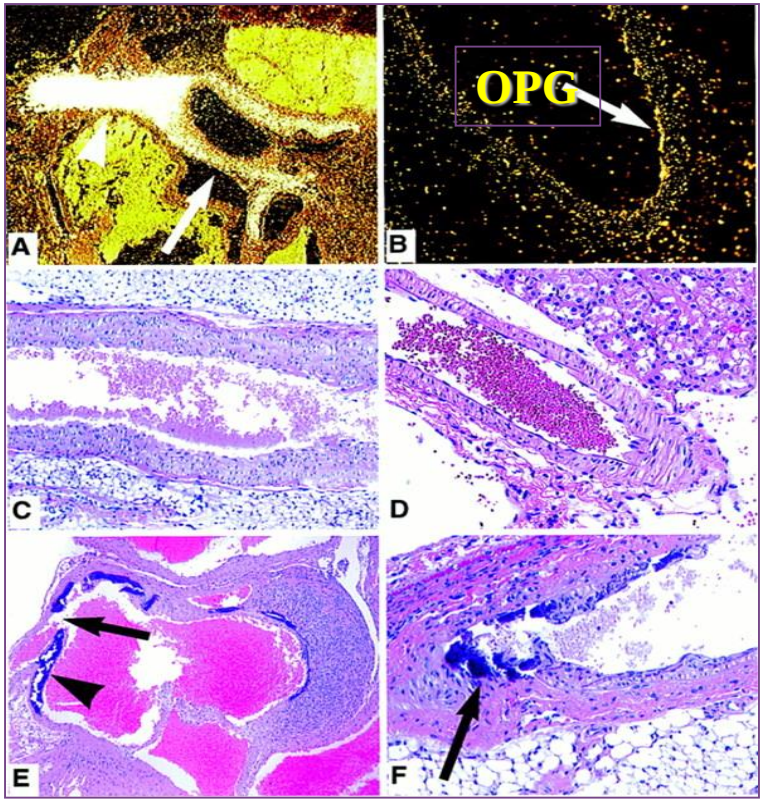
Panizo, S. et al. Circ Res 2009;104:1041-1048

OPG is expressed in major arteries and in VSM cells

Aorta **Renal artery**

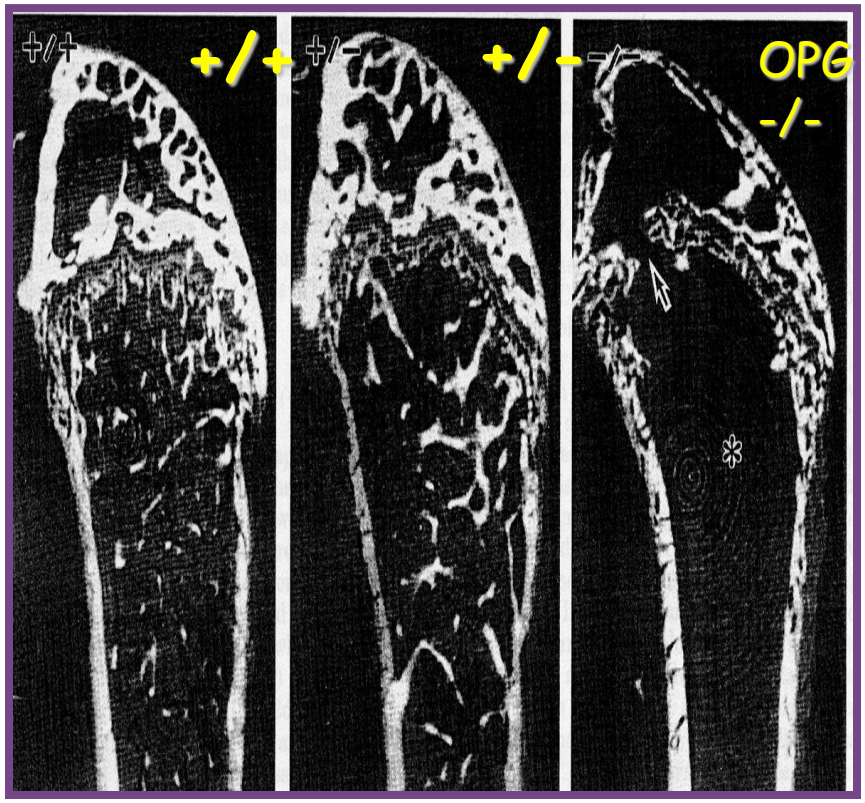
OPG+/+

OPG-/-



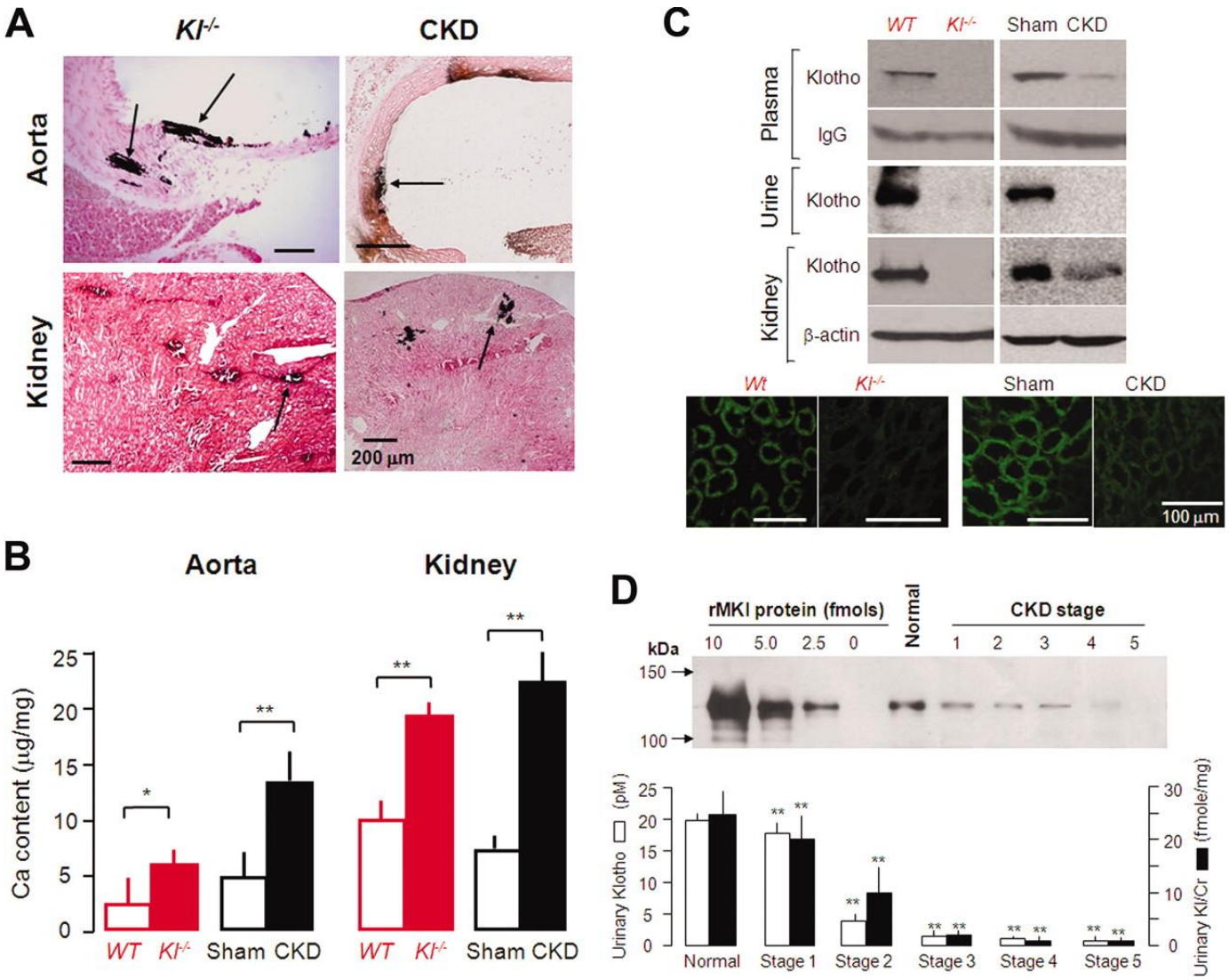
Bucay et al. , Genes Dev. 1998 1;12(9):1260-8.

OPG knock-out induces severe osteoporosis



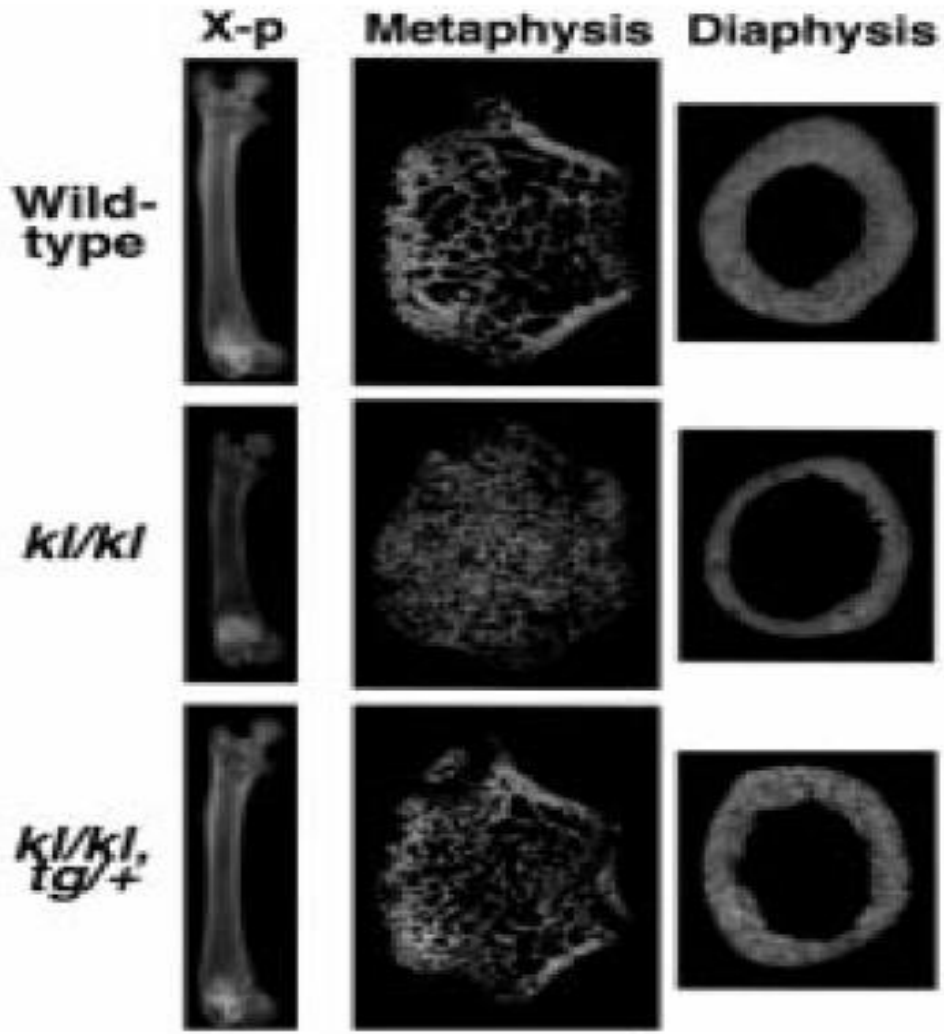
Mizuno et coll.BBRC, 247, 1998

Klotho levels are reduced in CKD mice and CKD patients, and soft tissue calcification is observed in CKD mice.



Hu M C et al. JASN 2011;22:124-136

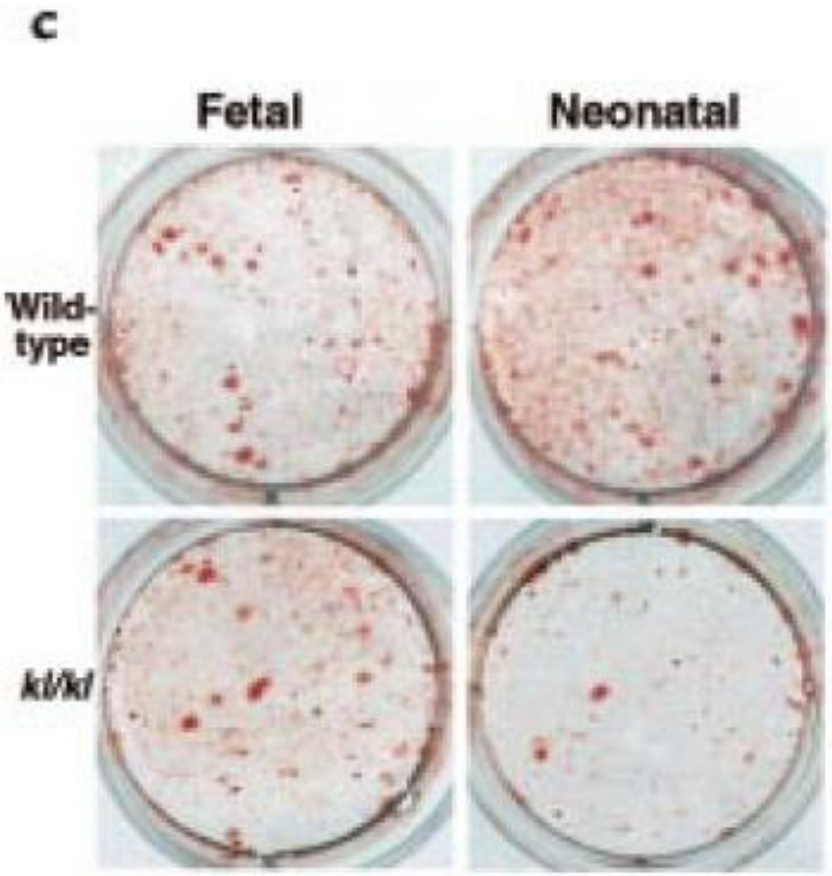
Plain X-rays and CT of the femur of 7 weeks old wild typeand kl-/kl- mice

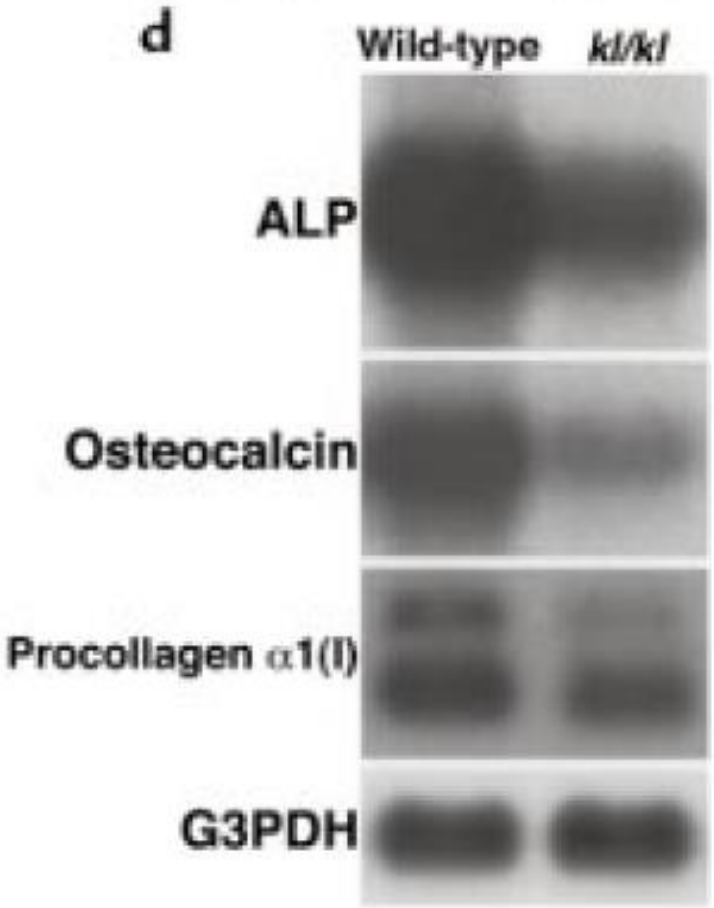


Short length and porosis

Kawaguchi H et al. JCI 1999;104:229-37

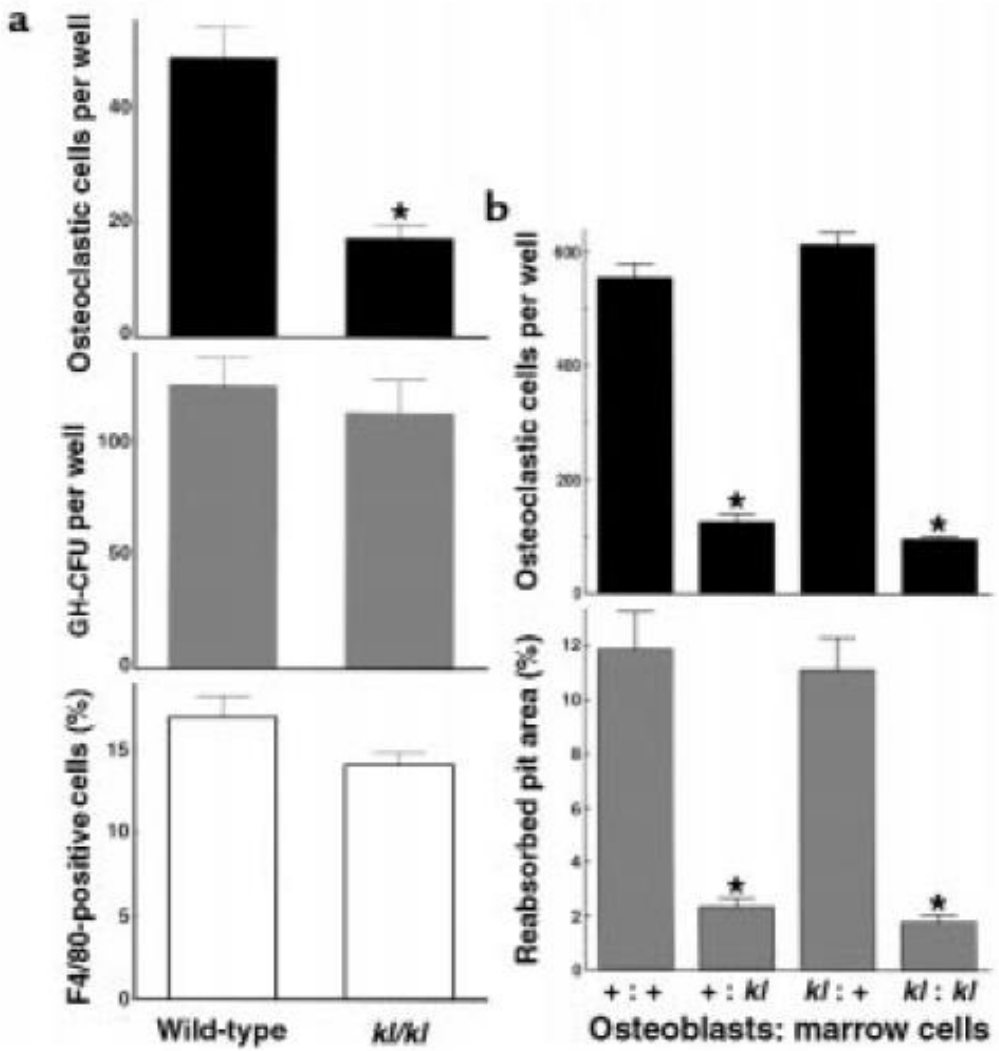
Cultures of osteoblasts from wild-type and kl-/kl- mice





Kawaguchi H et al. JCI 1999;104:229-37

Osteoclastogenesis in ex vivo bone marrow cell culture (a), and in the coculture of osteoblastic and bone marrow cells



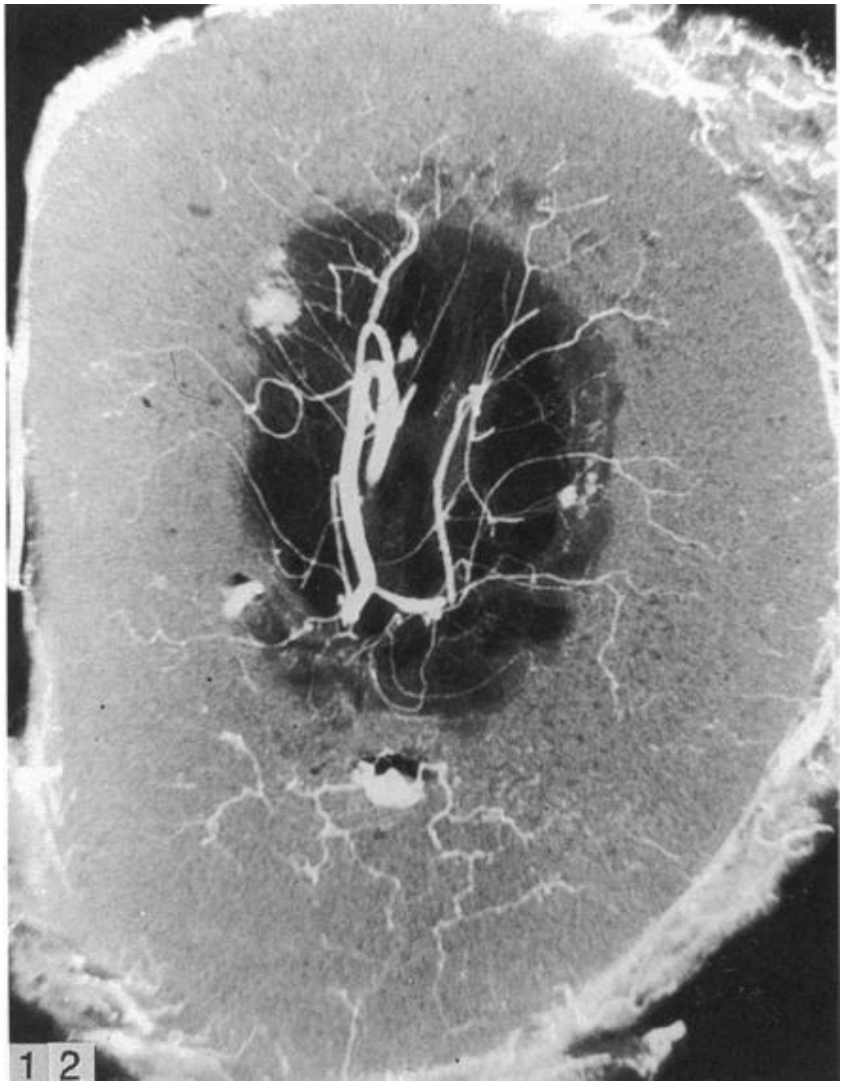
Kawaguchi H et al. JCI 1999;104:229-37

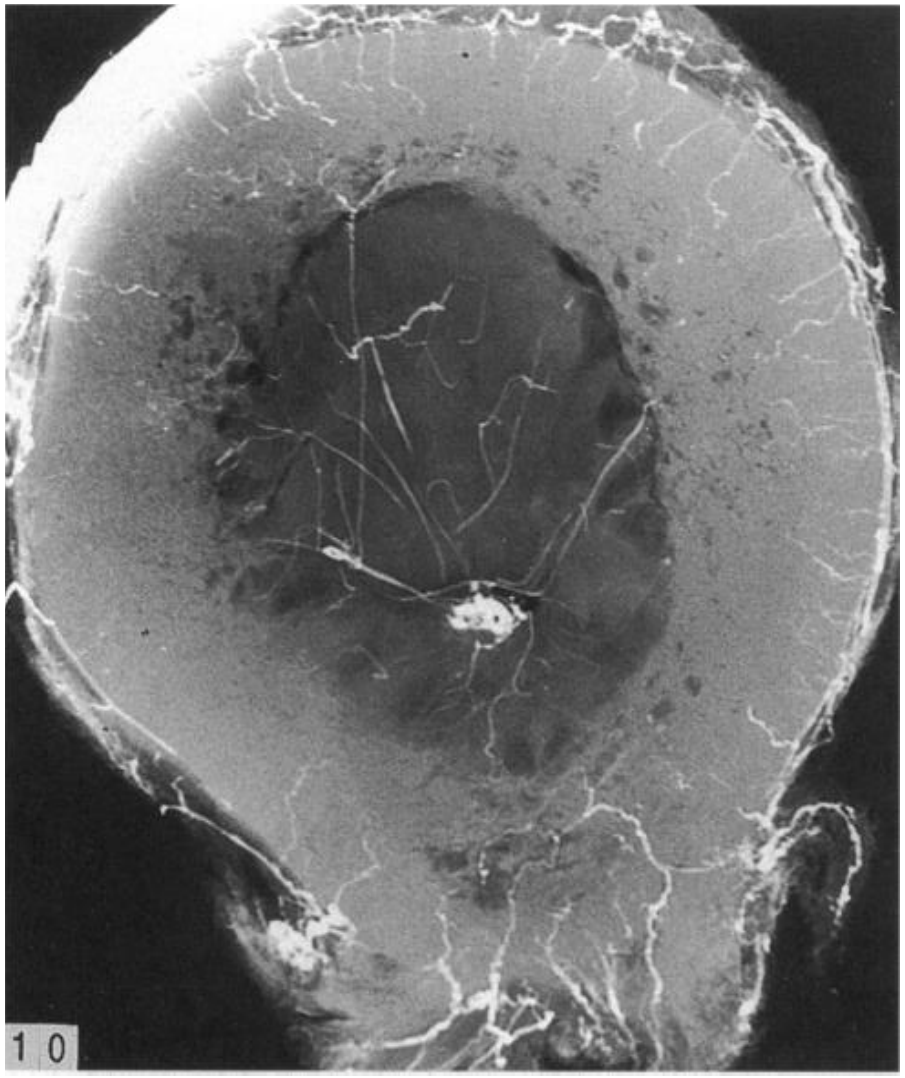
Possible Mechanisms Associating Bone and Arterial Disorders

- Common factors: inflammation, tobacco, lipid disorders, estrogen deficiency, aging, diabetes, oxidative stress, …
- Generalized arterial disease involving bone arteries – reduced circulation to bone
- Primary bone alteration affecting systemic arteries

Blood supply to human femoral femoral diaphysis in youth and senescence

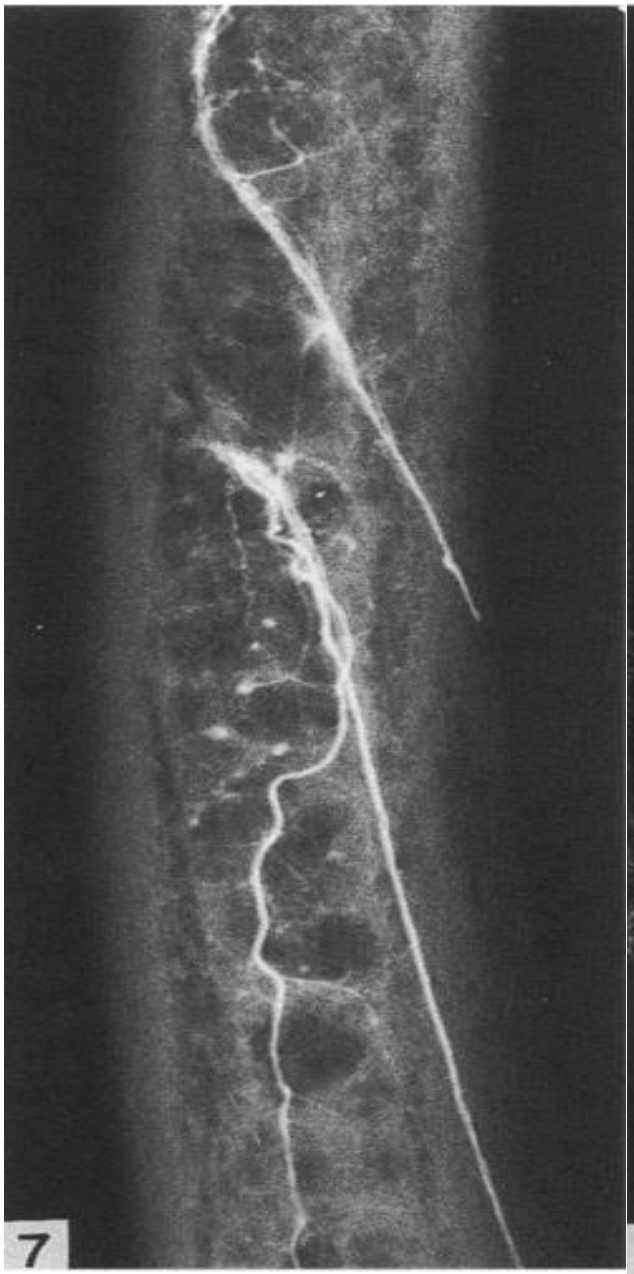
Bridgeman G and Brookes M J Anat 1996;188:611-21

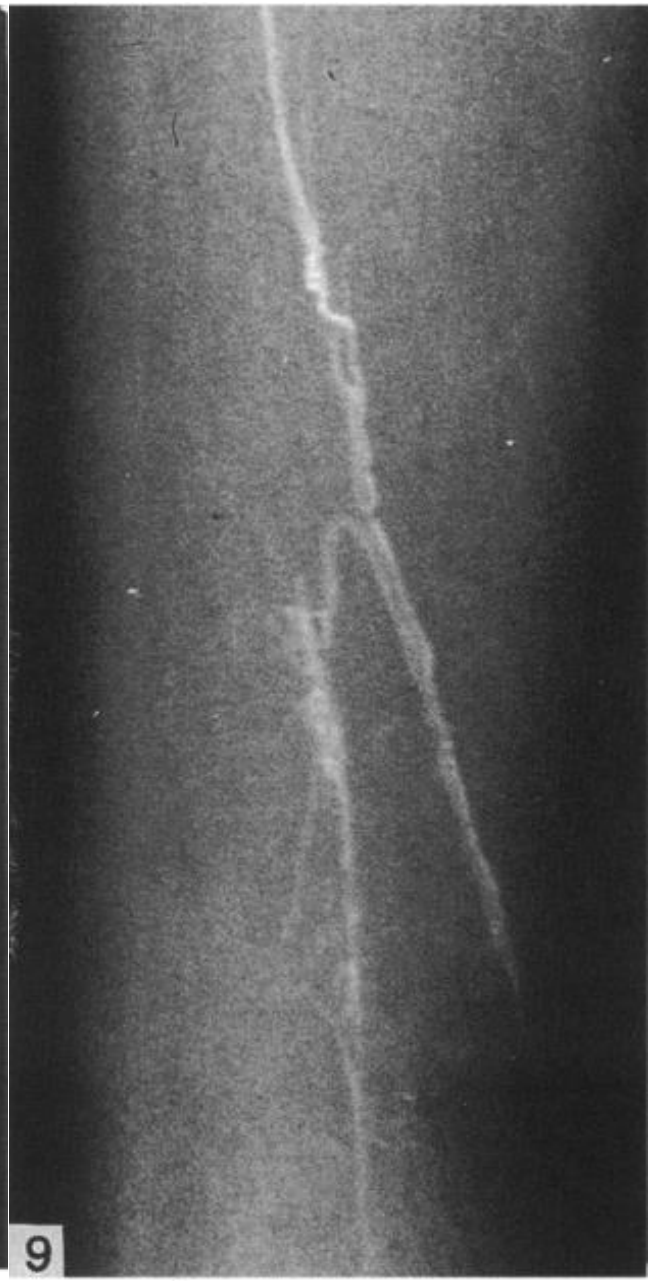




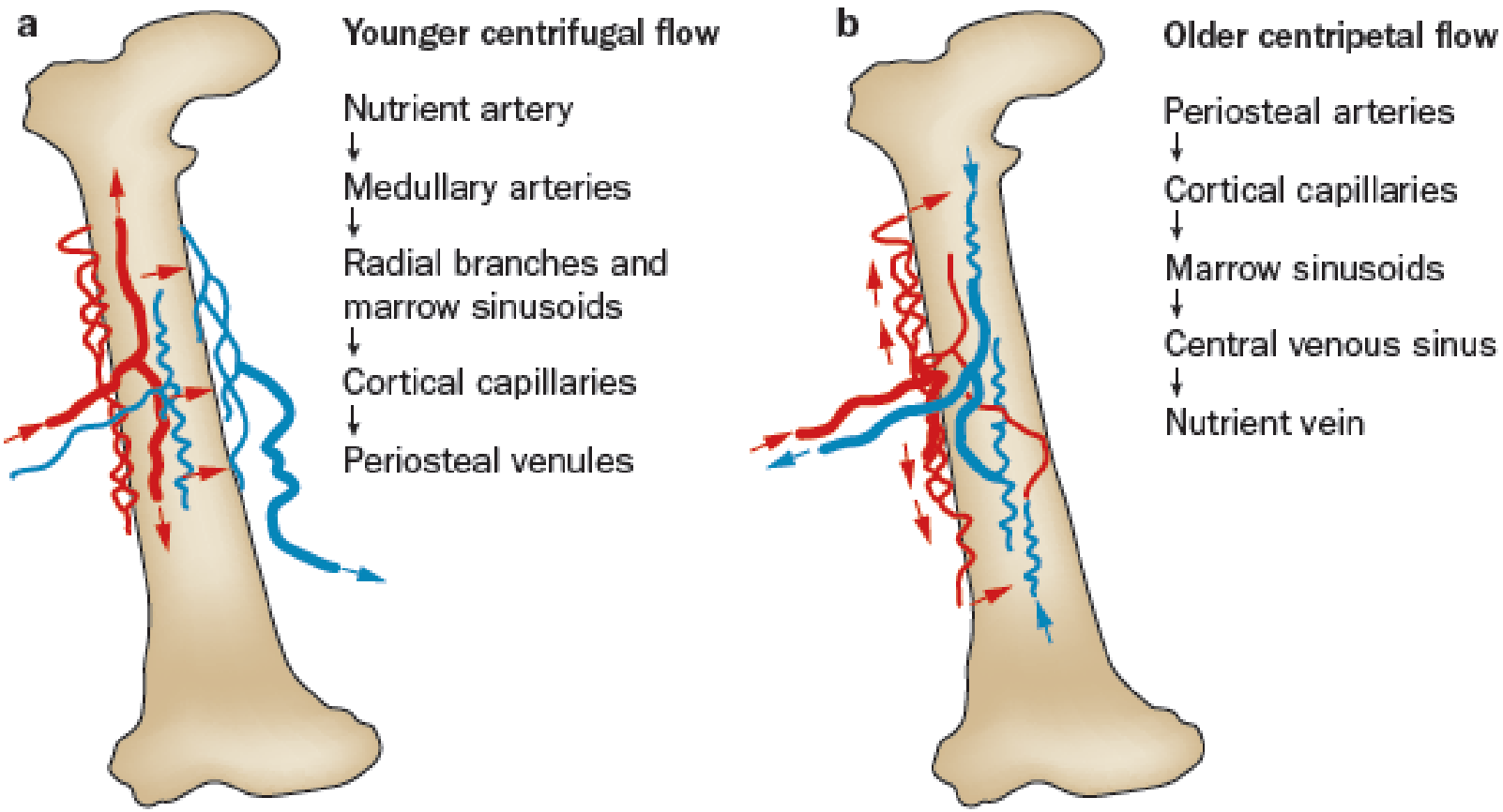
42 years – robust medullary arteries
Who supply the cortex

65 yrs – dominant periosteal arterial supply
The marrow is ischemic

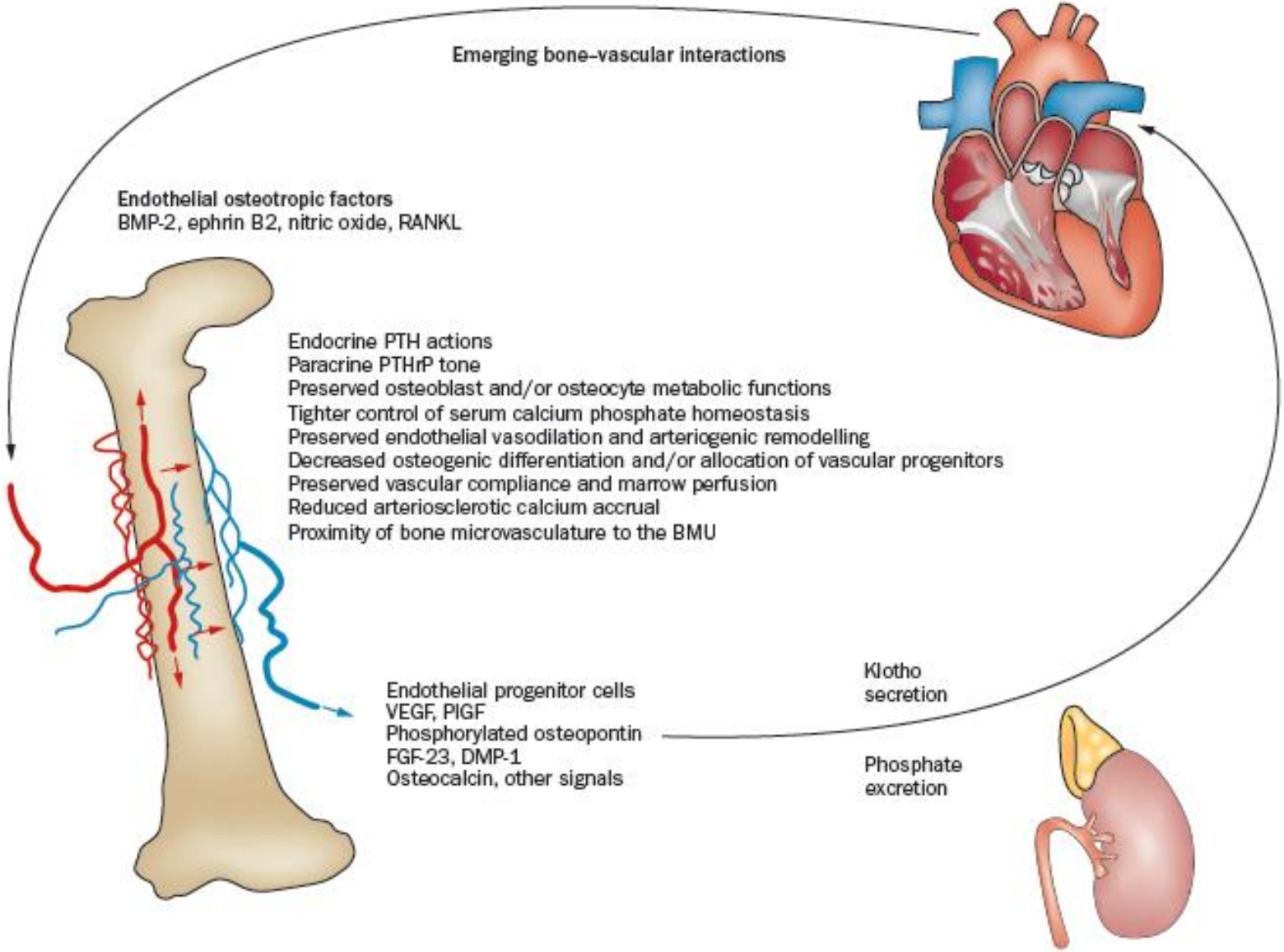




Bridgeman G and Brookes M J Anat 1996;188:611-21



Thomson B and Towler D Nature Review endocrinology 2012;8:529-543



Thomson B and Towler D Nature Review endocrinology 2012;8:529-543

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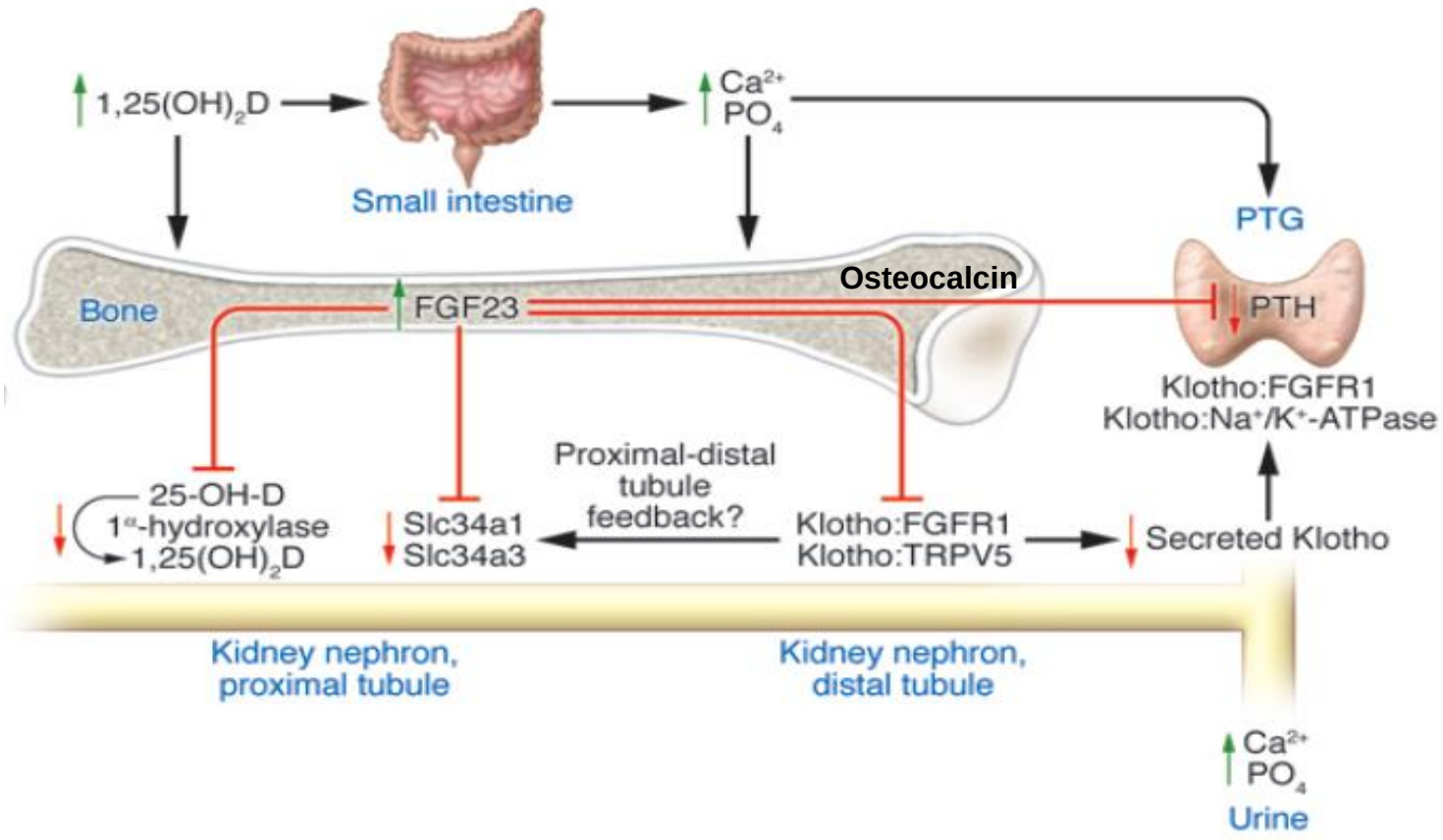
Endocrine functions of bone in mineral metabolism regulation

L. Darryl Quarles

J. Clin. Invest. **118**:3820–3828 (2008)



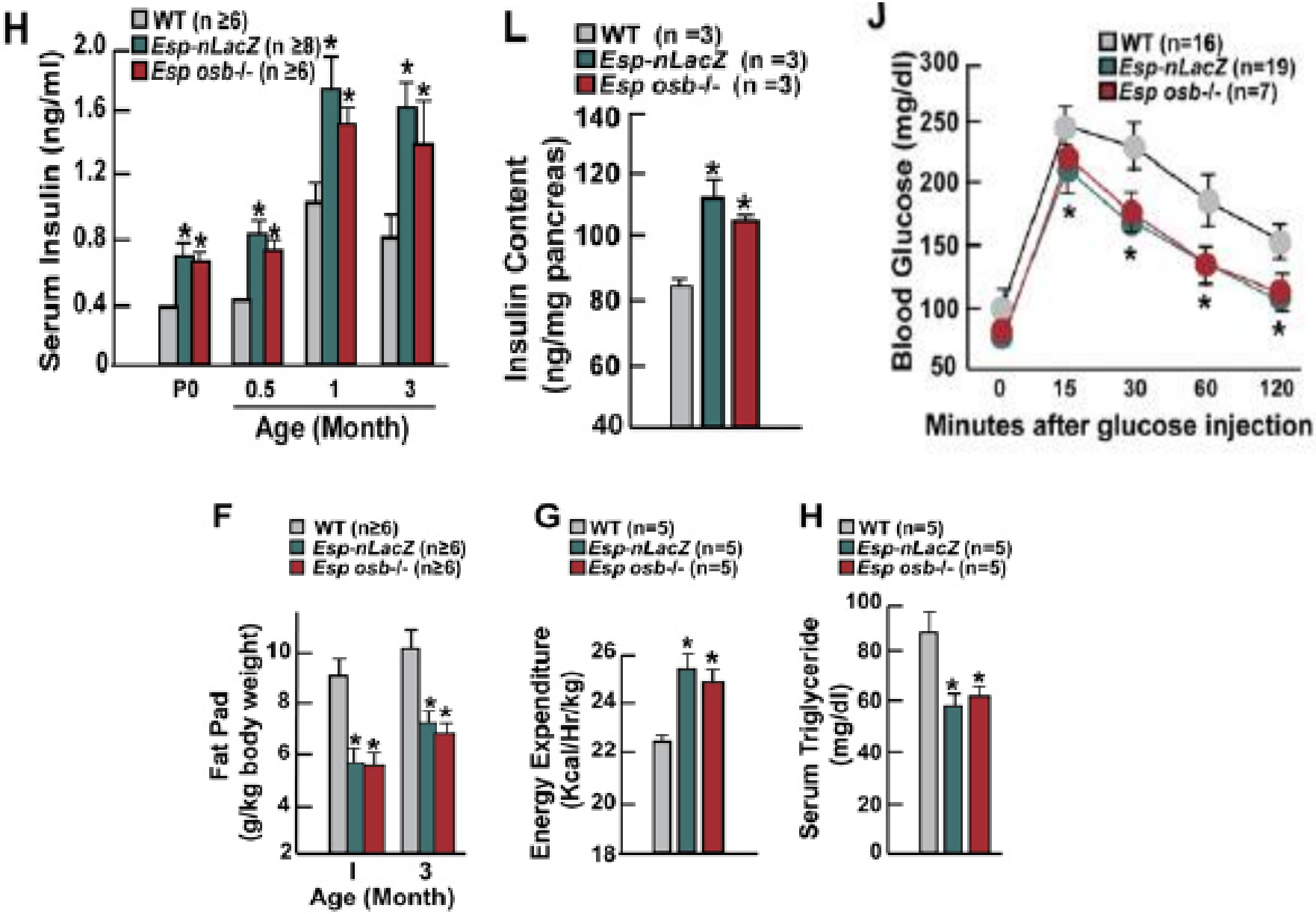
Endocrine Regulation of Energy Metabolism by the Skeleton

Na Kyung Lee,[1] Hideaki Sowa,[1] Eiichi Hinoi,[1] Mathieu Ferron,[1] Jong Deok Ahn,[3] Cyrille Confavreux,[1] Romain Dacquin,[4] Patrick J. Mee,[5] Marc D. McKee,[6] Dae Young Jung,[7] Zhiyou Zhang,[7] Jason K. Kim,[7] Franck Mauvais-Jarvis,[8] Patricia Ducy,[2] and Gerard Karsenty[1,*]

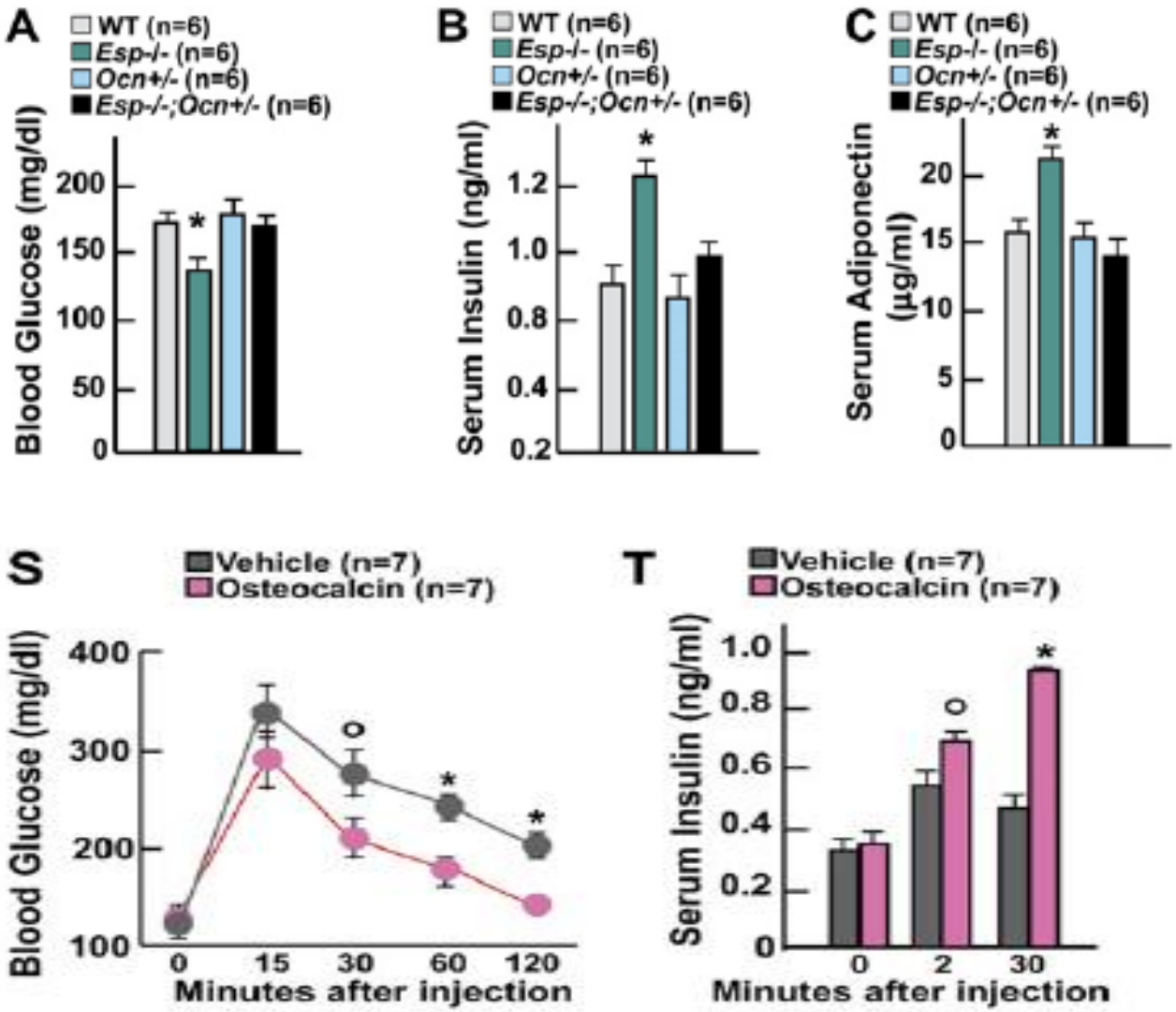
Lee NK et al. Cell 230:456-69,2007

SUMMARY

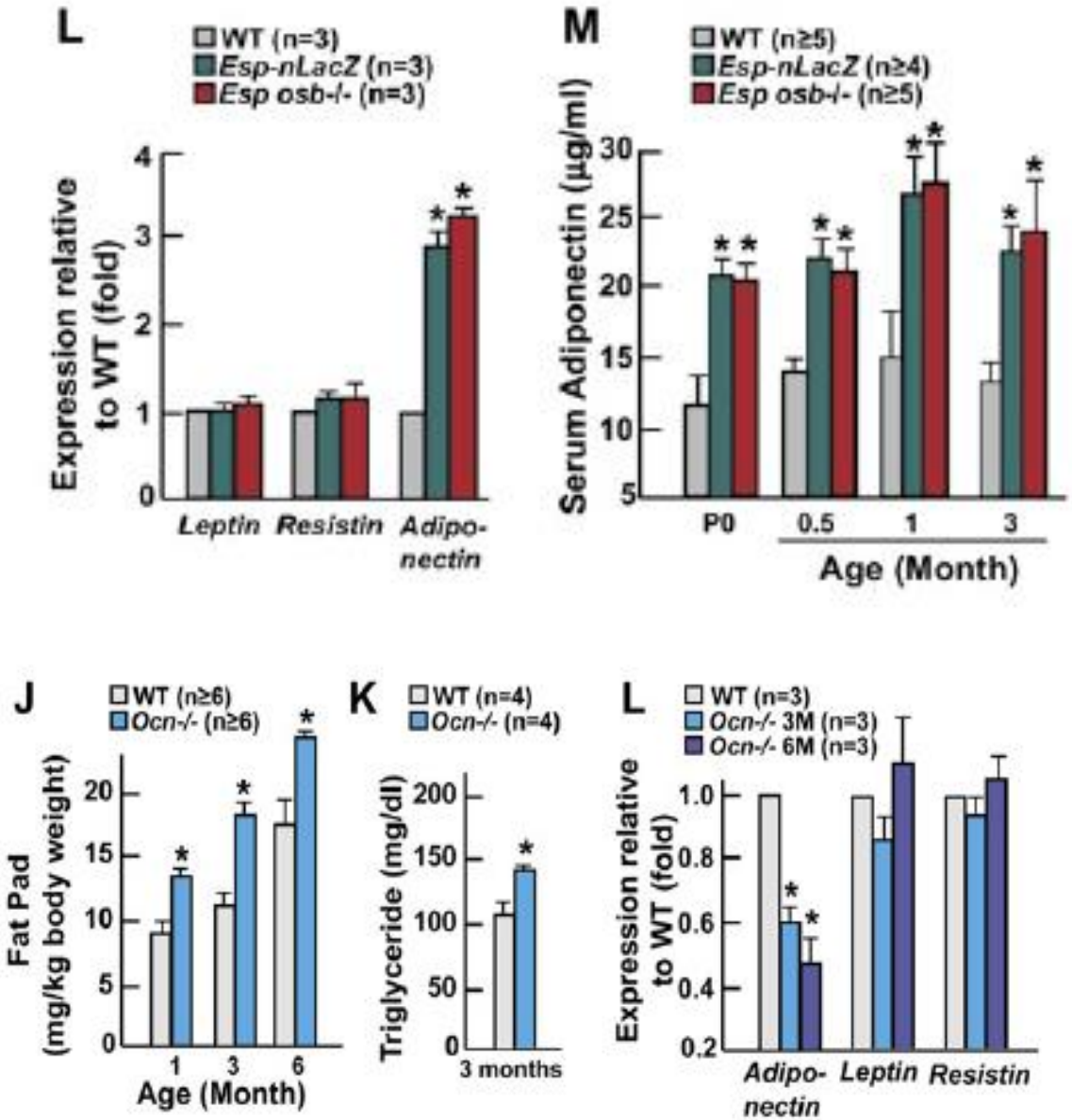
The regulation of bone remodeling by an adipocyte-derived hormone implies that bone may exert a feedback control of energy homeostasis. To test this hypothesis we looked for genes expressed in osteoblasts, encoding signaling molecules and affecting energy metabolism. We show here that mice lacking the protein tyrosine phosphatase OST-PTP are hypoglycemic and are protected from obesity and glucose intolerance because of an increase in β-cell proliferation, insulin secretion, and insulin sensitivity. In contrast, mice lacking the osteoblast-secreted molecule osteocalcin display decreased β-cell proliferation, glucose intolerance, and insulin resistance. Removing one *Osteocalcin* allele from OST-PTP-deficient mice corrects their metabolic phenotype. Ex vivo, osteocalcin can stimulate *CyclinD1* and *Insulin* expression in β-cells and *Adiponectin*, an insulin-sensitizing adipokine, in adipocytes; in vivo osteocalcin can improve glucose tolerance. By revealing that the skeleton exerts an endocrine regulation of sugar homeostasis this study expands the biological importance of this organ and our understanding of energy metabolism.



Lee NK et al. Cell 230:456-69,2007



Lee NK et al. Cell 230:456-69,2007



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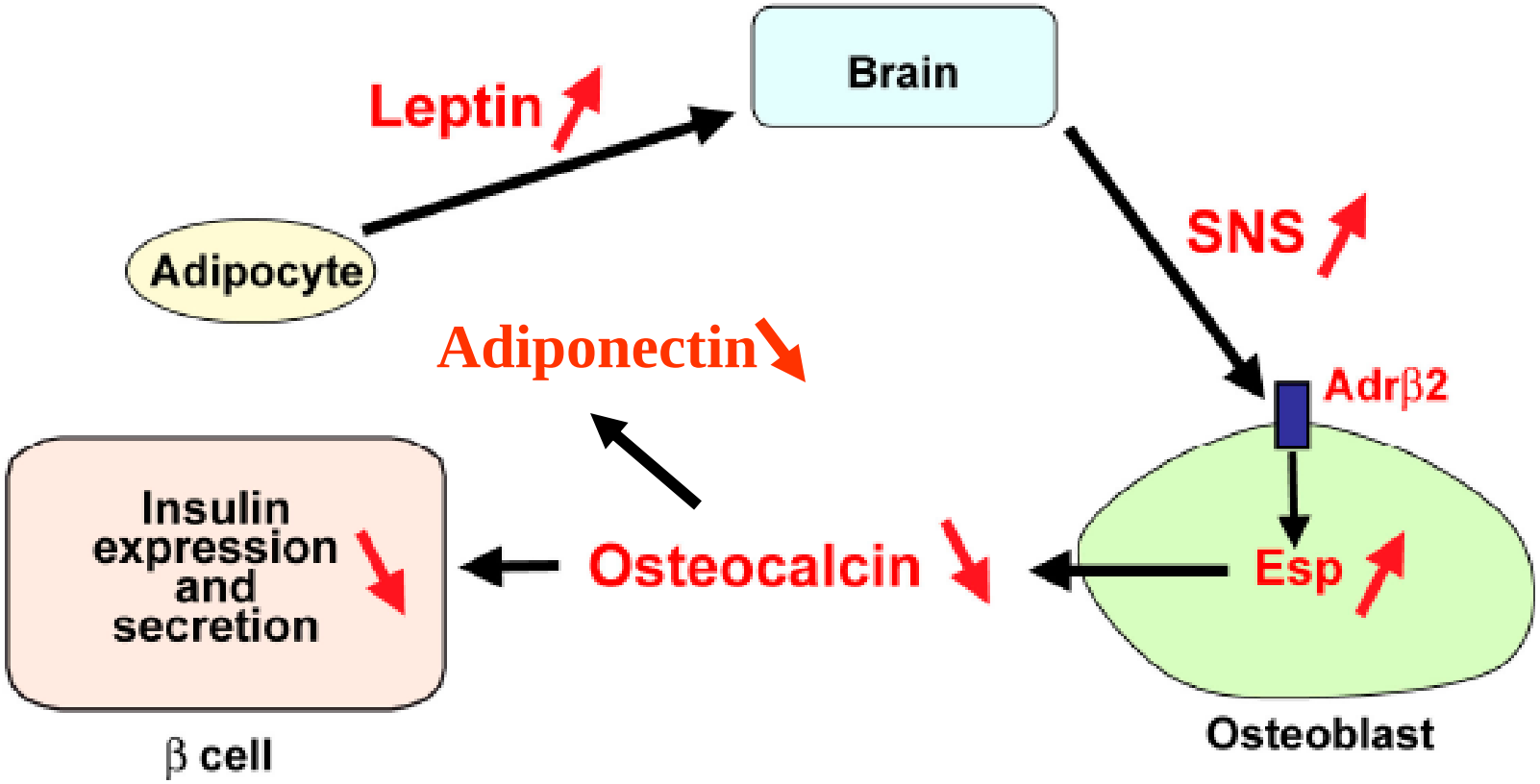

Hinoi E et al. JCB 2008;143:1235-42

The relationship between adipokines, osteocalcin and bone quality in chronic kidney disease

Nephrol Dial Transplant (2009) 1–8

Justine Bacchetta[1,2,3], Stéphanie Boutroy[2,3], Fitsum Guebre-Egziabher[3,4,5], Laurent Juillard[3,4,6], Jocelyne Drai[3,5,7], Solenne Pelletier[2,4], Michel Richard[3,7], Anne Charrié[3,6,8], Marie Christine Carlier[3,7], Roland Chapurlat[2,3], Maurice Laville[3,4,6] and Denis Fouque[3,4,5]

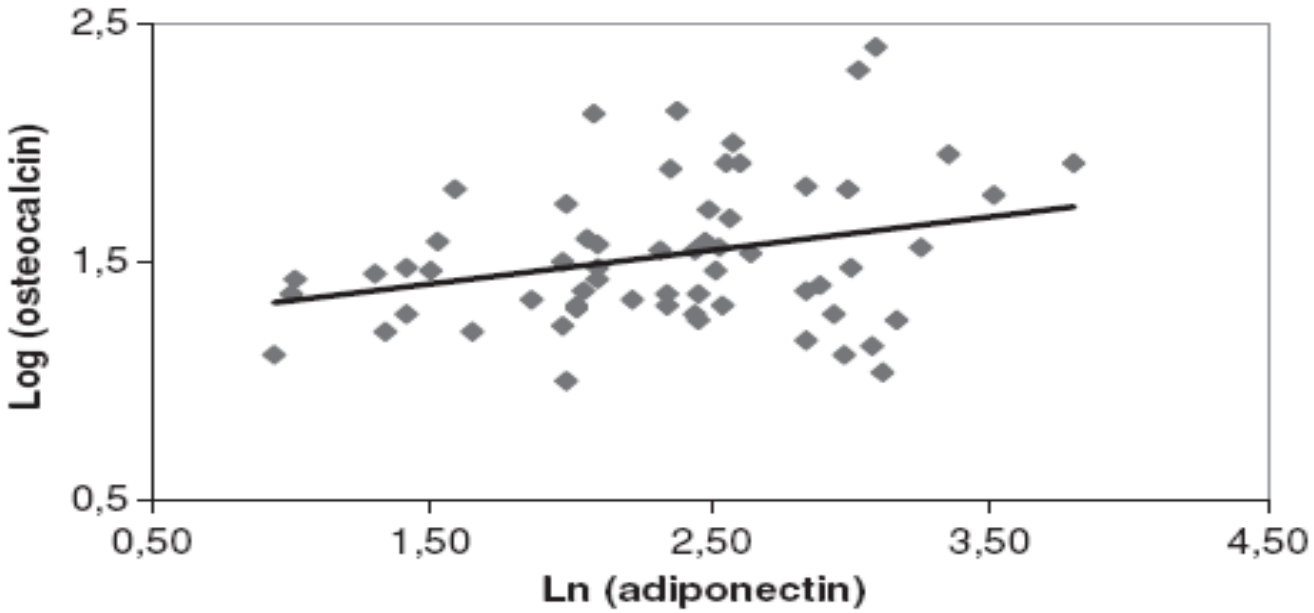

Fig. 2. The osteocalcin–adiponectin relationship ($r = 0.29$; $P = 0.021$).

Adiponectin and AMP kinase activator stimulate proliferation, differentiation, and mineralization of osteoblastic MC3T3-E1 cells

Ippei Kanazawa, Toru Yamaguchi*, Shozo Yano, Mika Yamauchi, Masahiro Yamamoto and Toshitsugu Sugimoto

Conclusion: Taken together, this study suggests that adiponectin stimulates the proliferation, differentiation, and mineralization of osteoblasts via the AdipoR1 and AMP kinase signaling pathways in autocrine and/or paracrine fashions.

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BMC Cell Biology 2007, **8**:51

Low Plasma Adiponectin Levels Predict Progression of Coronary Artery Calcification

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Background—Circulating adiponectin levels are lower in men than in women and lower in advanced coronary artery disease, obesity, and type 2 but not type 1 diabetes. However, it is not known whether low adiponectin levels predict development of atherosclerosis independently of other cardiovascular risk factors.

Methods and Results—Progression of coronary artery calcification (CAC) over an average of 2.6 years (range, 1.6 to 3.3) was assessed in a cohort of patients with type 1 diabetes and nondiabetic subjects 19 to 59 years of age. In this nested case-control substudy, plasma adiponectin levels were measured in 101 cases with significant CAC progression and in 205 controls. Controls were oversampled on the basis of age, gender, diabetes status, and presence of baseline CAC. In conditional logistic regression adjusted for baseline CAC volume and other significant predictors of CAC progression, adiponectin levels were inversely related to progression of CAC in diabetic (OR, 0.47; 95% CI, 0.24 to 0.94) and nondiabetic (OR, 0.15; 95% CI, 0.05 to 0.40 for a doubling in adiponectin levels) subjects. Adjustment for additional cardiovascular risk factors did not change this association. In conditional logistic regression models by quartiles of plasma adiponectin levels, the probability value for trend was statistically significant for all participants ($P<0.001$) and nondiabetic participants ($P<0.001$) and was borderline for type 1 diabetics ($P=0.08$).

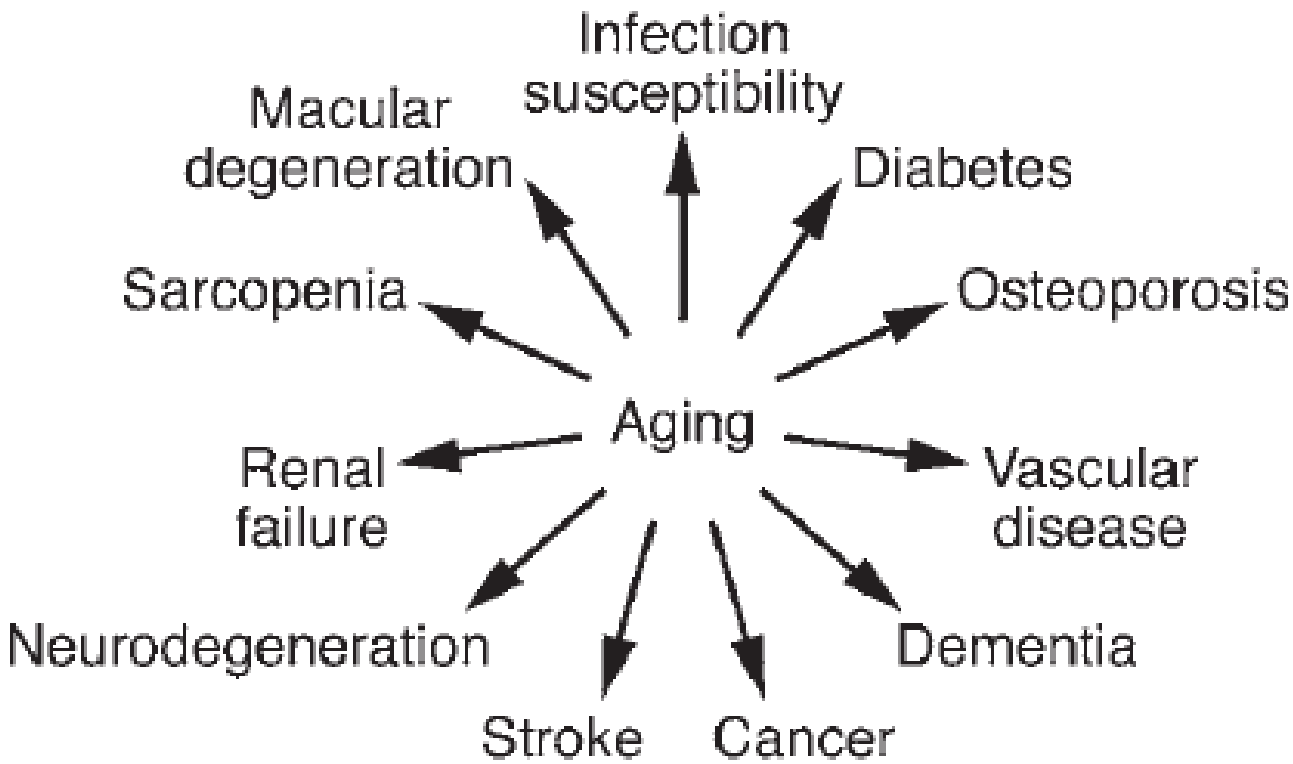
Conclusions—Low plasma adiponectin levels are associated with progression of CAC in type 1 diabetic and nondiabetic subjects independently of other cardiovascular risk factors. **(*Circulation*. 2005;111:747-753.)**

Table 1. Hot Research Topics in the Bone-Vascular Axis: A Few Important Biological Pathways That Implicate a Causal Connection Between Bone and Vascular Diseases

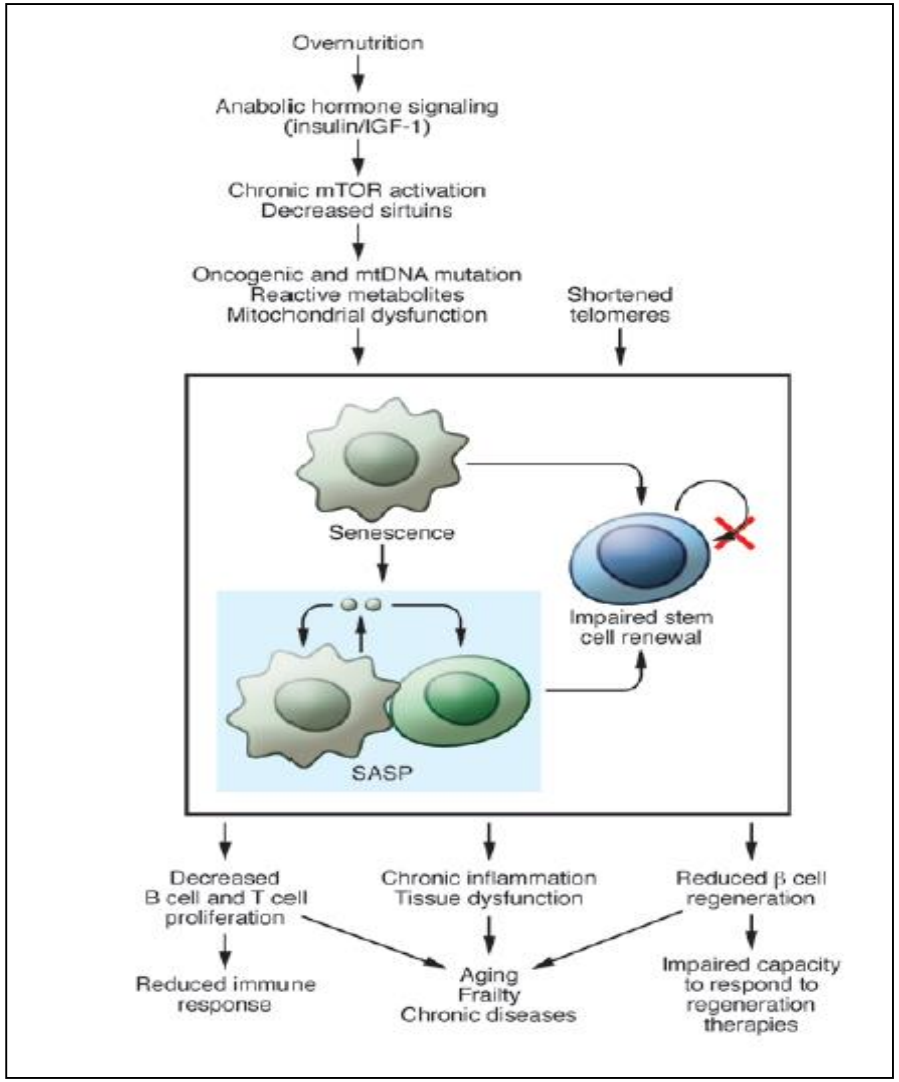
Pathway	Association With Bone Metabolism/Disease	Association With Vascular Disease
Inflammation	Reduces bone mineral density	Colocalizes with increased vascular and valve calcification
OPG/RANK/RANKL axis	Regulates osteoblast-osteoclast interplay and bone mineral density	Debated role during atherogenesis; OPG is a known inhibitor of vascular calcification
Calciprotein particles/fetuin-A mineral complexes	Hypothetical increase in circulating levels during bone-resorptive disorders	Inhibitory effect of fetuin-A on ectopic mineralization; controversial participation of calciprotein particles in vascular calcification
FGF-23/Klotho axis	Regulation of vitamin D and phosphate metabolism; deficiency associated with osteopenia	Lack of Klotho and FGF-23 associated with vascular calcification
Circulating calcifying cells	Mobilized by fractures and contribute to bone healing	Participation in atherosclerotic vascular/valve calcification

OPG indicates osteoprotegerin; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; and FGF-23, fibroblast growth factor-23.

Fadini GP et al Circulation 2012;125:2772


Infection susceptibility
Macular degeneration
Diabetes
Sarcopenia
Osteoporosis
Aging
Renal failure
Vascular disease
Neurodegeneration
Dementia
Stroke
Cancer

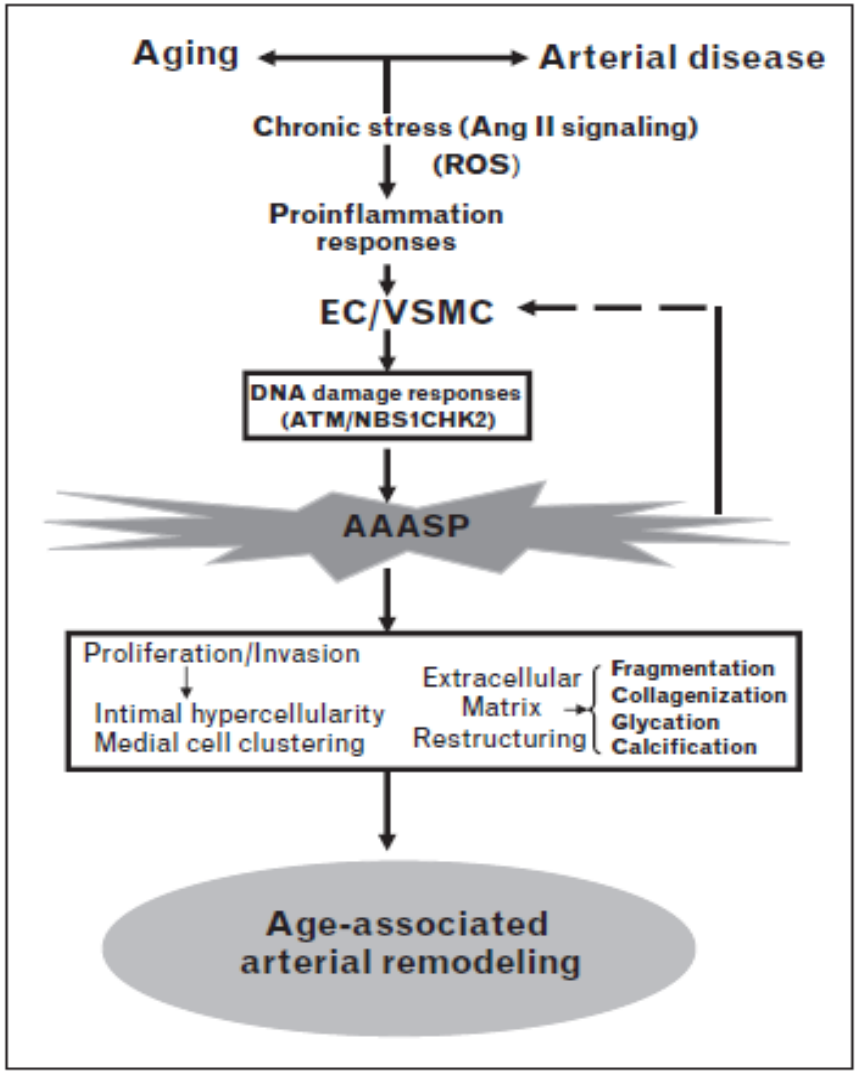
Molecular pathways implicated in aging and Senescence-associated secretory phenotype



SASP - senescence associated secretory phenotype

Newgard CB and Sharpless NE JCI 2013;123

Molecular and cellular mechanisms for arterial remodelling in aging



AAASP – age-associated arterial secretory phenotype

Wang M. et al. Current Opin Nephrol Hypertens 2010