Arterial Calcifications and functions

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Cyclic strain inhibits switching of smooth muscle cells to an osteoblast-like phenotype

Janeta Nikolovski,* Byung-Soo Kim, and David J. Mooney*−5,2

Chronic cyclic strain down-regulates bone-specific genes in smooth muscle tissues.
Correlation between age and arterial calcium (Ca) concentration in the aorta and internal iliac artery in nonuremic control subjects

Comparison of aortic total calcium score with coronary total calcium score

AGE vs AoPWV

A. ESRD patients – green circles
   Control subjects -- red circles

B. ESRD aortic calcific. Positive – ESRD orange circles
   ESRD aortic calcific. negative – red circles

London GM et al Blood purification 2013
Molecular and cellular mechanisms for arterial remodelling in aging

Molecular pathways implicated in aging and Senescence-associated secretory phenotype

SASP – senescence associated secretory phenotype

Newgard CB and Sharpless NE  JCI 2013;123

AAASP – age-associated arterial secretory phenotype

Nuclear lamina and posttranslational processing

Cleavage impossible in HG for deletion of 50 amino acids
In the cleavage site with accumulation of progerin and decreased lamin A
Lamin A/C in fibroblasts from Young, Old and HGPS patients
Oxidative stress reduces ZMPSTE24/FACE1 activity and accumulates Prelamin A
Prelamin A accumulation promotes VSMC calcification

Liu Y et al Circ.Res 2013
Prelamin A accumulated in calcified VSMCs both in vivo and in vitro.

Klotho levels are reduced in CKD mice and CKD patients, and soft tissue calcification is observed in CKD.
Dietary and genetic evidence of phosphate toxicity accelerating mammalian aging. Ohnishi M and Razzaque MS Faseb 2010 24;3562

DKO klotho/Napi double knock-out
NPD normal phosphate diet
HPD high phosphate diet
Effects of low phosphate (PD) and low vitamin D diet (DD) on aortic calcifications in FGF23 knock-out (KO) mice

Worman HJ et al. JASN 2007;18:2116
Effects of aldosterone and spirolactones on PIT1 expression
Effects of aldosterone and spirolactones on calcification factors

![Graphs showing the effects of aldosterone and spirolactones on calcification factors.](image-url)

Figure 2. Role of NF-κB in mediating the nongenomic effects of aldosterone in cardiovascular and kidney disease.

TWEAK: TNF-like weak inducer of apoptosis


Moreno JA et al JASN 2011;22:1315
Advanced glycation end products induce calcification of VSMC through RAGE/p38MAPK and upregulation of Runx2

Infliximab downregulates osteogenic BMP2-Msx2-Wnt programs in Ldlr-/- mice fed HFD

A

Relative Aortic mRNA Accumulation
Percent Chow Fed Control (18S Normalized)

BMP2

Msx2

Wnt3a

Wnt7a

Runx2

Dkk1

C

p = 0.034 (two tail)

Aortic Calcium Content
(µg/mg aortic dry weight)

HFD + Vehicle

HFD + Infliximab

HFD-high fat diet
Figure 1. Unadjusted Kaplan–Meier Cumulative-Event Curves for Coronary Events among Participants with Coronary-Artery Calcium Scores of 0, 1 to 100, 101 to 300, and More Than 300. $P < 0.001$ for all.
Correlation between calcium score and degree of stenosis is weak

- Calcification is not equivalent to coronary stenosis

Gottlieb. JACC 2010
Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation

Wael E. Shaalan, MD, Hongwei Cheng, MD, PhD, Bruce Gewertz, MD, James F. McKinsey, MD, Lewis B. Schwartz, MD, Daniel Katz, MD, Dindcai Cao, PhD, Tina Desai, MD, Seymour Glagov, MD, and Hisham S. Bassiouny, MD, Chicago, Ill

Conclusions: Symptomatic plaques are less calcified and more inflamed than asymptomatic plaques. Regardless of clinical outcome, a strong inverse correlation was found between the extent of carotid plaque calcification and the intensity of plaque fibrous cap inflammation as determined by the degree of macrophage infiltration. Carotid plaque calcification is associated with plaque stability, and is a potential spiral CT in vivo quantitative marker for cerebrovascular ischemic event risk. (J Vasc Surg 2004;40:262-9.)
FIGURE 5. Lumped average percentages of nodules remaining attached for both control (triangles) and BGP-treated (squares) cultures ($n=9$, $p=0.47$) as a function of shear stress ($\text{dyn/cm}^2$).
What is the role of coronary calcium measurement by fast CT scan in asymptomatic patients with high CHD risk?

The Committee does not advise CAC measurement in this selected patient stratum as they are already judged to be candidates for intensive risk reducing therapies based on current NCEP guidelines.

Is there evidence that coronary calcium measurement is better than other potentially competing tests in intermediate risk patients for modifying cardiovascular disease risk estimate?

In general, CAC measurement has not been compared to alternative approaches to risk assessment in head-to-head studies. This question cannot be adequately answered from available data.
Paradigm change

- Cardiology switched from anatomy to function
- Recommendations 2004: tight stenosis with lumen reduction > 50%
- Recommendations 2010: functional evaluation FFR: <0.8 ischemia (functional flow rate)

\[
FFR = \frac{Q_s}{Q_{max}} = \frac{(P_d-P_v)/R}{(P_a-P_v)/R} = \frac{P_d}{P_a}
\]

- Pa – aortic pressure
- Pd – wire pressure
Correlation between aortic calcification score and aortic PWV in ESRD patients

\[ r = 0.754 \]
\[ P < 0.0001 \]

Pannier et al. Artery 2007
Smaller fraction of \( \text{O}_2 \) available for metabolism

**Oxygen Limitation Model**

- **Blood Velocity**
- **Capillary Transit Time**
- **\( \text{O}_2 \) Extraction**
- **Smaller Fraction of \( \text{O}_2 \) Available for Metabolism**

Normally 2-3 seconds

(Breit M et al KI 2012)
Pressure wave analysis

- measured pressure wave
- forward/incident pressure wave
- reflected pressure wave
- pulse wave velocity

PWV 6 m/s Young subjects

PWV 12 m/s Old subjects
Role of increased central aortic and pulse pressures in the increase of cardiovascular events

Decreased Coronary Artery Perfusion Pressure in Diastole
- Increased risk of MI

Increase in left ventricular load (LV load) accelerates increase in LV mass
- Increased risk of LV hypertrophy

Increase in the central pulse pressure that drives cerebral blood flow
- Increased stroke risk
- Increased chronic kidney disease.
Reflection coefficient = RefP / Forward P
Aortic PWV and White matter Hyperdensities (WMH)

The interaction of PWV and age in relation to the global composite cognitive score

Henskens et al. Hypertension 2008;52:1120

Angiotensin II and Aldosterone Regulate Gene Transcription Via Functional Mineralocorticoid Receptors in Human Coronary Artery Smooth Muscle Cells
Iris Z. Jaffe and Michael E. Mendelsohn
Circ Res. 2005;96:643-650
TNF-α directly activates Msx2-Wnt signaling with subsequent ALP induction.
Working model for arterial media calcification
Recorded aortic pressure wave

Recorded peripheral pressure wave at reflection sites

The time interval of pressure wave to and back from reflection sites

Forward and backward pressures are in phase: no time interval

$P_{\text{backward (reflected)}}$ $P_{\text{forward}}$

Briet M et al KI 2012
Klotho regulates phosphate uptake and calcium content and VSMC dedifferentiation
By regulating Pit-1 and 2 and Runx2
Summary and Conclusion

While several serious efforts to understand the cost-effectiveness of CAC measurement have been made, the Committee felt that models were not, and could not be, sufficiently well grounded in data to offer results that could be used for medical decision making or establishing policy at this time.
Figure 2 | Effect of pyrophosphate (PPI) on aortic calcium content in uremic rats. Rats were fed a diet consisting of 2.5% protein, 1.06% phosphorus, 0.92% calcium, and 0.75% adenine for 4 weeks and received sodium PPI in Hank’s buffered saline or saline alone by daily intraperitoneal injection of 5 ml/kg. Each point represents a single animal.
Calciphylaxis Registry:
Role of calcification inhibitors: serum Fetuin-A

Courtesy of Dr. Brandenburg V
Calciphylaxis
role of calcification inhibitors: serum MGP


→ Very low levels of circulating calcification inhibitors fetuin / MGP: cause versus effect?
Calciphylaxis Registry: PTH levels [pg/mL]

Calciphylaxis registry
March 2012

NDT educational online survey
February 2012

→ Relevant differences between perception and registry data regarding PTH levels

Courtesy of Dr. Brandenburg V
Role of vitamin K antagonist usage and CUA

Hayashi M et al; NDT 2012

A case–control study of calciphylaxis in Japanese end-stage renal disease patients

Table 4. Results of using the multivariate logistic regression model to identify predictors of calciphylaxis at the time the diagnosis of calciphylaxis was made

<table>
<thead>
<tr>
<th></th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin therapy</td>
<td>0.013</td>
<td>10.1 (1.63-62.7)</td>
</tr>
<tr>
<td>Serum albumin (for each 1 g/dL decline)</td>
<td>0.003</td>
<td>12.7 (2.35-68.6)</td>
</tr>
<tr>
<td>Plasma glucose (for each 100 mg/dL increment)</td>
<td>0.309</td>
<td>0.99 (0.97-1.01)</td>
</tr>
</tbody>
</table>

→ Clear association between VKA usage and calciphylaxis: Causality?
Calciphylaxis Registry: outcome

Courtesy of Dr. Brandenburg V

Product-Limit Survival Estimates

STS-treatment
No STS-treatment
STS unknown

50% 516 days

FUThiosulfat
Calciphylaxis Registry:
Reported treatment strategies ("state-of-the-art" therapy)

“nephrological”

- Stop calcium containing phosphate-binders
- Use calcium-free phosphate binders
- Intensify dialysis (duration, frequency)
- Lower dialysis bath calcium (e.g. to 1.00 mmol/L)
- Stop active Vitamin D
- Give Sodium thiosulfate (STS) i.v.

bisphosphonates
parathyroidectomy
cinacalcet
FFP infusion
Causes of death (%) for all dialysis patients by age

20–44 years
Rate: 95 deaths/10^3 patient years

45–64 years
Rate: 173 deaths/10^3 patient years

+65 years
Rate: 341 deaths/10^3 patient years

USRDS. AJKD, 1998
A Kaplan–Meier estimate of patient survival following a diagnosis of CUA compared to control cases matched for age, modality, duration and year of commencing renal replacement therapy. Survival was significantly worse in patients diagnosed with CUA (HR for death 2.9, 95% CI 1.2–6.9, $P=0.017$).
Correlation Between CCr (C-G formula) and Aortic PWV

$\text{Aortic PWV (m/s)}$

$\text{Creatinine clearance (mL/min/m}^2\text{)}$

$r = -0.30$

$P<0.0001$

Bortolotto et al KI 2001
Calciphylaxis Registry: Co-medication [%]

Vitamin D  |  PB  | Coumadins  | Cinacalcet  | ESA

% 80  |  70  |  60  |  50  |  40

Courtesy of Dr. Brandenburg V
**Calciphylaxis outcome literature data**

A Kaplan–Meier estimate of patient survival following a diagnosis of CUA compared to control cases matched for age, modality, duration and year of commencing renal replacement therapy. Survival was significantly worse in patients diagnosed with CUA (HR for death 2.9, 95% CI 1.2–6.9, \( P = 0.017 \)).
Calciphylaxis Registry: Patient characteristics
April 2012 (5.5 yrs)

N = 144 pts
N = 27 pts / yr

median age: 69 yrs
age range: 20 – 88 yrs

60% female
40% male

Courtesy of Dr. Brandenburg V
Calciphylaxis Registry: underlying Renal Disease [%]

- vast majority of registered pts are hemodialysis pts

- HD [78%]
- PD [5%]
- CKD [9%]
- no CKD [4%]
- transplant [4%]
Activation of Vascular Smooth Muscle Parathyroid Hormone Receptor Inhibits Wnt/\(\beta\)-Catenin Signaling and Aortic Fibrosis in Diabetic Arteriosclerosis

Su-Li Cheng, Jian-Su Shao, Linda R. Halstead, Kathryn Distelhorst, Oscar Sierra, Dwight A. Towler

**Rationale:** Vascular fibrosis and calcification contribute to diabetic arteriosclerosis, impairing Windkessel physiology necessary for distal tissue perfusion. Wnt family members, upregulated in arteries by the low-grade inflammation of “diabetes,” stimulate type I collagen expression and osteogenic mineralization of mesenchymal progenitors via \(\beta\)-catenin. Conversely, parathyroid hormone (PTH) inhibits aortic calcification in low-density lipoprotein receptor (LDLR)-deficient mice fed high fat diabetogenic diets (HFD).

**Objective:** We sought to determine the impact of vascular PTH receptor (PTH1R) activity on arteriosclerotic Wnt/\(\beta\)-catenin signaling in vitro and in vivo. We generated SM-caPTH1R transgenic mice, a model in which the constitutively active PTH1R variant H23R-caPTH1R is expressed only in the vasculature.

**Methods and Results:** The caPTH1R inhibited Wnt/\(\beta\)-catenin signaling, collagen production, and vascular smooth muscle cell proliferation and calcification in vitro. Transgenic SM-caPTH1R;LDLR\(^{+/−}\) mice fed HFD develop diabetes, with no improvements in fasting serum glucose, cholesterol, weight, body composition, or bone mass versus LDLR\(^{+/−}\) siblings. SM-caPTH1R downregulated aortic Col1A1, Runx2, and Nox1 expression without altering TNF, Mx2, Wnt7a/b, or Nox4. The SM-caPTH1R transgene decreased aortic \(\beta\)-catenin protein accumulation and signaling in diabetic LDLR\(^{+/−}\) mice. Levels of aortic superoxide (a precursor of peroxide that activates pro-matrix metalloproteinase 9 and osteogenic signaling in vascular smooth muscle cells) were suppressed by the SM-caPTH1R transgene. Aortic calcification, collagen accumulation, and wall thickness were concomitantly reduced, enhancing vessel distensibility.

**Conclusions:** Cell-autonomous vascular smooth muscle cell PTH1R activity inhibits arteriosclerotic Wnt/\(\beta\)-catenin signaling and reduces vascular oxidative stress, thus limiting aortic type I collagen and calcium accrual in diabetic LDLR-deficient mice. *(Circ Res. 2010;107:271-282.)*
### Aldosterone Regulates Endogenous Gene Expression in Human Vascular Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Molecular Function</th>
<th>Microarray Fold Change</th>
<th>Q-RT-PCR Fold Change</th>
<th>Q-RT-PCR P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen type I</td>
<td>Pro-fibrotic, vascular calcification</td>
<td>3.3</td>
<td>2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>Pro-fibrotic</td>
<td>1.6</td>
<td>1.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Parathyroid hormone receptor 2 (PTH2)</td>
<td>Vascular calcification</td>
<td>2.1</td>
<td>3.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone morphogenetic protein 2 (BMP2)</td>
<td>Vascular calcification</td>
<td>2.1</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Vascular calcification</td>
<td>1.5*</td>
<td>2*</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-16 (lymphocyte chemoattractant factor)</td>
<td>Immune response/inflammation</td>
<td>1.7</td>
<td>1.8</td>
<td>0.056</td>
</tr>
<tr>
<td>Cytotoxic T-lymphocyte–associated protein 4 (CTLA4)</td>
<td>Immune response/inflammation</td>
<td>1.9*</td>
<td>2.1*</td>
<td>0.076</td>
</tr>
<tr>
<td>Aldosterone synthase</td>
<td>Aldosterone production</td>
<td>Not detected</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>NaK ATPase</td>
<td>Renal ion transport</td>
<td>Unchanged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial sodium channel (ENaC)</td>
<td>Renal ion transport</td>
<td>Not detected</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Downregulation of Bone Morphogenetic Protein 4 Expression in Coronary Arterial Endothelial Cells
Role of Shear Stress and the cAMP/Protein Kinase A Pathway

Anna Csiszar, Nazar Labinskyy, Kira E. Smith, Araceli Rivera, Erik N.T.P. Bakker, Hanjoong Jo,
Jason Gardner, Zsuzsanna Orosz, Zoltan Ungvari

Objective—Bone morphogenetic protein 4 (BMP-4) is a transforming growth factor β family member cytokine that exerts proinflammatory effects on the endothelium and is likely to play a role in atherogenesis. Recent studies suggested that atheroprotective levels of shear stress control endothelial BMP-4 expression; however, the underlying mechanisms remained unknown.

Methods and Results—We found that shear stress downregulated BMP-4 expression in human and rat coronary arterial endothelial cells (CAECs) as well as in cultured mesenteric arterioles, although it had no effect on the expression of BMP-2, a related cytokine. In human coronary arterial endothelial cells, 8-bromo-cAMP, the adenylate cyclase activator forskolin, or a cAMP-dependent protein kinase (PKA) activator effectively decreased BMP-4 expression, mimicking the effects of shear stress. Indeed, shear stress induced the nuclear translocation of PKA-α, and inhibition of PKA attenuated the effects of shear stress and forskolin on BMP-4 expression. RNA decay assay and BMP-4 promoter-driven luciferase reporter gene assay showed that cAMP regulates BMP-4 expression at the transcriptional level.

Conclusions—Laminar shear stress and the cAMP/PKA pathway are important negative regulators of BMP-4 expression in the vascular endothelium. Because BMP-4 elicits endothelial activation and dysfunction, hypertension, and vascular calcification, inhibition of BMP-4 expression by shear stress and the cAMP/PKA pathway is likely to exert antiatherogenic and vasculoprotective effects. (Arterioscler Thromb Vase Biol. 2007;27:776-782.)
The arterial wall is a heterogeneous material.
Cellular aging is accelerated by TOR

Age-related diseases are linked to TOR

Animal lifespan is shortened by TOR

The concept of TOR-centric aging

Inhibitors of TOR to prolong healthy life

Inhibitors of TOR are approved for human use
BMP- Msx2-Wnt signaling in arterial calcifications
AOP *** (Accentuated Osteogenic Program)

TNFα, IL-1, RANKL, ROS, glucose, AGEs, oxLDL→TLR2/4

MGP, Noggin Chordin, Gremlin, Follistatin, shear stress

I-Smad

DNA binding of Smad4/R SmadR:Runx2

Wnt3a Wnt7a

Wnt antagonists
dickkopf 1 SOST

LRP5/LRP6 Frizzled receptors

β-catenin

DNA binding of Smad: β-catenin β-catenin:TCF:LEF

OPN, OPG Fetuin, Adiponectin, Pyrophosphates.

Matrix remodelling Elastolysis

Arterial Calcification

BMP2/4

BMPR I/II

Smad4 R-Smad

Runx2, Msx2 ALP, Osterix

Osteochondrogenic Gene program

MMP9, MMP2 Cathepsin

Doxycyclin MMP antagonists

Adapted from Shao et al. Ann NY Acad Sci And Boström et al. Circ Res 2011
Figure 5. Inflammatory cytokines decrease Klotho expression in cultured tubular cells in an NFκB-dependent manner. TWEAK (A) and TNFα (B) decrease Klotho mRNA.
Briet M et al. KI 2011
Briet M et al. KI 2011
NC/DC – necrotic core/dense calcium
SAP – stable ang.pect

Kono K et al. KI 2012;82:344-51
Prognostic factors for mortality evaluated by coronary scan:
Degree of stenosis; Proximal localisation; Number of vessels
A broad population-based strategy of CAC screening does not appear to be warranted. It is not clear whether it is reasonable to consider CAC scanning in persons whose global risk assessment places them in the intermediate-risk category or whether the findings from such testing will lead to a beneficial increase in the intensity of treatment. This issue needs to be addressed in future trials focusing on clinical outcomes and cost-effectiveness.
The concept of fractional flow reserve (FFR). $Q_{s \text{max}}^s$ : hyperemic myocardial blood flow in the presence of a stenosis, $Q_{N \text{max}}^N$ : normal hyperemic myocardial blood flow, $P_d$: distal coronary pressure, $P_a$: aortic pressure, $P_v$: venous pressure, $R$: hyperemic myocardial resistance.

\[
\text{FFR} = \frac{Q_{s \text{max}}^s}{Q_{N \text{max}}^N} = \frac{(P_d - P_v)/R}{(P_a - P_v)/R} = \frac{P_d}{P_a}
\]
Consequences of stiffening

**Oxygen Limitation Model**

↑ Blood Velocity

↓ Capillary Transit Time

↓ \( O_2 \) Extraction

⇒ Smaller Fraction of \( O_2 \) Available for Metabolism

Normally 2-3 seconds

(NOT capillary recruitment)
Aortic PWV and White matter Hyperdensities (WMH)

Henskens et al Hypertension 2008;52:1120