

# In vivo and in vitro studies of arterial calcifications in diabetes

The association to osteoprotegerin and the influence of hormonal and metabolic factors

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## ABSTRACT

This PhD project was carried out at the Research Laboratory for Biochemical Pathology, Institute of Clinical Medicine, Aarhus University Hospital, from 2002 to 2005.

Diabetes is associated with an increased prevalence of cardiovascular disease. Arterial media calcification appears to be a strong independent predictor of cardiovascular mortality in diabetes. The evidence from clinical observations, animal models and biochemical studies suggest that arterial media calcifications share same features with osteogenesis. However, the mechanism behind the medial calcification is unclear. This study is based on the hypothesis that arterial media calcifications are the consequences of a series of diffuse matrix alterations in the arterial wall caused by metabolic and hormonal disorder in diabetes. Bone related protein may occur in altered amount in the arterial wall in diabetes and during the calcification. Consequently, the levels of osteoprotegerin (OPG) in aortic samples from diabetic individuals are analysed. Furthermore, the acute influences of metabolic and hormonal factors on the production of OPG from vascular cells and the long term effect of insulin on vitro-induced calcification in human vascular smooth muscle cells (VSMCs) are also estimated.

ELISA, histochemistry and RT-PCR are used to demonstrate the expression of bone-related protein and component of extracellular matrix (hyaluronan). The accumulation of OPG is increased in tunica media from diabetic samples, but no differences between diabetic and non-diabetic subjects are observed in tunica intima. Human VSMCs produce approximately 30 times more OPG than human umbilical vein endothelial cells. The production of OPG is decreased after insulin or TGF- $\beta$  treatment, whereas TNF- $\alpha$  or IL-1 $\beta$  promotes synthesis of OPG in a time and dose dependent manner in both protein and mRNA levels. The progress of calcification induced by  $\beta$ -glycerophosphate is liable of time and accelerated at high dose of insulin (1000 $\mu$ U/mL). Increased activity of alkaline phosphatase (ALP, marker for osteoblast early differentiation), up-regulated bone sialoprotein (BSP, major non-collagenous protein in the extracellular matrix of bone) and high level of hyaluronan are found in calcified cells. No alterations in OPG levels are observed in early calcification, but a reduction of OPG occurs in strong calcification.

Increased OPG amount in the tunica media in diabetes may be a part of generalized matrix changes; the calcification in VSMCs is, at least, partially a cell-mediated process with upregulated bone proteins, which can be accelerated with insulin at high concentrations. The study suggests that OPG is a potent regulator of vascular calcification, which may be important for the elucidation of diabetic macroangiopathy.