# The calcium paradox

# Summary

In this thesis, I investigated the role of vitamin K in both the skeletal and vascular system. I focussed on processes involved in vascular calcification (VC) and bone mineralisation aiming to glean the molecular mechanisms behind it. This thesis entails a literature review followed by *in vivo* and *in vitro* studies trying to elucidate these aspects.

**Chapter 2** elaborates on the role of vitamin K on calcium supplementation in the bone-vascular axis. Calcium supplements are generically prescribed to combat bone-loss and increase bone strength. We discuss the impact of vitamin K and vitamin K dependent proteins (VKDPs) in bone and vascular. Data in postmenopausal women suggest that calcium supplementation significantly correlates with cardiovascular morbidity and morbidity yet provides little benefit on bone. This phenomenon is described as the "calcium paradox" and describes the simultaneous decline in bone calcium mineral content with the deposition of calcium at extra-osseous sites. Dialysis patients are also often prescribed calcium supplements in the form of phosphate binders (PBs) and their use is associated with increased VC. Initiation and progression of VC in dialysis patients correlates with impaired mineral metabolism represented by elevated serum levels of phosphate. Any increase in calcium results in pathological calcium-phosphate precipitation. In chapter 1, we impose the supplementation of vitamin K in the dietary regimen. We provide a literature overview that shows that vitamin K supplementation can benefit both vasculature and bone, preventing calcium residing in soft tissues including arteries and improving skeletal parameters.

In **Chapter 3** we describe a novel preclinical *in vivo* model of vitamin K deficiency in CKD, representing the clinical situation of end-stage CKD patients. Using this model, we showed that the use of PB treatment is not sufficient to prevent ectopic calcification. However, combining PBs with high vitamin K2 supplementation, counteracting the vitamin K deficiency, strongly attenuates VC. This protective calcification effect is likely accomplished by the synergistic effect of combined PB treatment and vitamin K2 supplementation. Incorporation of vitamin K2 into PBs treatment also improved the levels of vascular matrix Gla-protein, a vitamin K dependent protein involved in the inhibition of vascular calcification. This study aids the understanding of the role of vitamin K in CKD and suggests the supplementation of vitamin K in the clinical setting.

In **Chapter 4** we investigated the role of vitamin K2 in bone formation. Vitamin K2 is known to exert a plethora of effects on the skeletal system resulting in increased bone mineral density. We report the successful differentiation of osteoblast using an iPSCs model of osteogenesis. Our data suggest a non-canonical function of VKDPs in early differentiation events, that can be modelled using iPSCs. We show that vitamin K2 induces an osteogenic cellular phenotype that might prove beneficial towards the development of tissue engineering solutions. This is accompanied by reducing oxidative stress diminishing the inflammatory response during bone formation. Our data provide the first account of several mechanisms of osteogenesis from iPSCs and the influence of vitamin K2 on the osteogenic processes.

**Chapter 5** provides insights into the inhibitory role of vitamin K2 and the calcium-channel blockers, amlodipine and gabapentin in VSMC mineralisation. We characterised the contractile, synthetic and chondrocyte-like VSMC phenotype and compared these to articular chondrocytes. Phenotypic modulation of VSMCs into synthetic and chondrocyte-like VSMCs is accompanied by loss of contractile markers, increased oxidative stress and greater calcium influx. Differentiated cells are more prone to mineralisation which is reflected by greater extracellular vesicle (EV) release. Chondrocytes release fewer EVs and calcify to a lesser extent than chondrocyte-like VSMCs. We identified several genes that might drive the calcification of VSMCs and account for the difference in the amount of mineralisation. Additionally, we provide evidence that vitamin K2 can inhibit VC *in vitro.* Finally, using our *in vitro*

model we demonstrate the inhibitory role of amlodipine and gabapentin in reducing VC of contractile, synthetic and chondrocyte-like VSMCs via a EV independent mechanism.