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Is vitamin D indispensable for Ca\textsuperscript{2+} homeostasis: lessons from knockout mouse models?

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doi:10.1093/ndt/gfi587
Advance Access publication 15 March 2005

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Role of vitamin D in the maintenance of Ca\(^{2+}\) balance

Calcium (Ca\(^{2+}\)) is undoubtedly one of the most tightly regulated ions in plasma of higher animals. Ca\(^{2+}\) is involved in the normal functioning of a wide variety of tissues and physiological processes which include bone formation, muscle contraction, blood clotting, nerve transmission and as a second messenger include bone formation, muscle contraction, blood clotting, nerve transmission and as a second messenger regulating the actions of many hormones. The homeostasis of Ca\(^{2+}\) is complex because the gastrointestinal tract, the bones and the kidneys all affect the Ca\(^{2+}\) balance. Furthermore, the vitamin D endocrine system is critical for the proper development and maintenance of this Ca\(^{2+}\) homeostatic system. Once vitamin D is absorbed from the diet or made in the skin by the action of sunlight, it is metabolized in the liver to 25-hydroxyvitamin D and then the kidney serves as the endocrine gland to produce the biologically active form of vitamin D. This active form of vitamin D, 1\(\alpha\),25-dihydroxyvitamin D\(_3\) [1,25(OH)\(_2\)D\(_3\)], is synthesized in the proximal tubule by the renal cytochrome P450 enzyme 25-hydroxyvitamin D\(_3\)-1\(\alpha\)-hydroxylase (1\(\alpha\)-OHase) [1]. The importance of this enzyme is underlined by severe disorders in Ca\(^{2+}\) homeostasis caused by mutations in the 1\(\alpha\)-OHase gene, including vitamin D-dependent rickets type I (VDDR-I). 1,25(OH)\(_2\)D\(_3\) remains the most potent active form of vitamin D known to date. The intestine and kidney are the main target organs for the action of this hormone. The biological effects of 1,25(OH)\(_2\)D\(_3\) on these target organs are mediated by both genomic and rapid post-transcriptional mechanisms [1]. 1\(\alpha\),25(OH)\(_2\)D\(_3\) transcriptionally controls the expression of a particular set of target genes mediated through a vitamin D receptor (VDR) acting as a ligand-inducible factor. Upon binding 1,25(OH)\(_2\)D\(_3\), the VDR undergoes a conformational change and forms a complex with a retinoid X receptor (RXR). This VDR–RXR complex binds to DNA elements in the promoter regions of target genes described as vitamin D response elements (VDREs). Binding to these VDREs controls the rate of gene transcription. The rapid response presumably utilizes another signal transduction pathway that is probably linked to putative plasma membrane receptors for 1,25(OH)\(_2\)D\(_3\), but its physiological role is not well understood.

Vitamin D-deficient knockout mice models

Targeted deletion of genes encoding 1\(\alpha\)-OHase [2,3] and of the nuclear VDR [4,5] have provided useful mice models of inherited human diseases such as VDDR-I (also known as pseudovitamin D-deficiency rickets; PDDR) and VDDR-II. Mice in which the 1\(\alpha\)-OHase gene was inactivated presented the same clinical phenotype as patients with PDDR, including undetectable levels of 1,25(OH)\(_2\)D\(_3\), rickets and secondary hyperparathyroidism [2,3]. On a normal diet, 1\(\alpha\)-OHase knockout mice have an average life span of 12±2 weeks [3,6]. Previous studies indicated that daily injections of 1,25(OH)\(_2\)D\(_3\) completely rescued these 1\(\alpha\)-OHase knockout mice [7]. Bone histology and histomorphometry confirmed that the rickets and osteomalacia were cured by this 1,25(OH)\(_2\)D\(_3\) supplementation. Blood biochemistry analysis revealed that the rescue treatment corrected the hypocalcaemia and secondary hyperparathyroidism. Interestingly, these 1\(\alpha\)-OHase knockout mice were also rescued by a Ca enriched diet (2% w/w) [8]. Dietary Ca normalized the hypocalcaemia, secondary hyperparathyroidism and the biomechanical properties of the bone tissue. Comparable results were obtained in VDR knockout mice from which the bone phenotype could be completely rescued by feeding the animals an enriched Ca\(^{2+}\), phosphorus and lactose diet, suggesting that vitamin D deficiencies can be rescued by dietary Ca in a vitamin D-independent manner [4]. Other studies have however indicated that exogenous Ca may not entirely compensate for 1,25(OH)\(_2\)D\(_3\) deficiency in mice and piglets [9,10]. In humans, beneficial effects of Ca infusions were reported in a child with hereditary resistance to 1,25(OH)\(_2\)D\(_3\) and alopecia [11]. Ca infusions may be an efficient alternative for the management of patients with this condition who are unresponsive to large doses of vitamin D derivatives. However, it is not completely clear whether dietary Ca is effective in humans, and studies are needed in which vitamin D-deficient subjects are treated with Ca enriched diets.

Gene products involved in high dietary Ca rescue of 1\(\alpha\)-OHase knockout mice

The kidney has a predominant role in maintaining the Ca\(^{2+}\) balance because it determines the final excretion of Ca\(^{2+}\) in the urine. Active Ca\(^{2+}\) reabsorption in the distal convoluted and connecting tubule comprises a sequence of processes involving apical Ca\(^{2+}\) entry via transient receptor potential channel V5 (TRPV5), translocation of Ca\(^{2+}\) through the cytosol by calbindins and extrusion over the basolateral membrane by the Na\(^{+}\)/Ca\(^{2+}\) exchanger (NCX1) and plasma membrane Ca\(^{2+}\) ATPase (PMCA1b) [12] (Figure 1). Recently, it was demonstrated that the expression of these renal Ca\(^{2+}\) transport proteins, with the exception of PMCA1b, is significantly downregulated in kidneys of 1\(\alpha\)-OHase knockout mice, which is in line with a diminished Ca\(^{2+}\) reabsorption capacity contributing to the development of the observed hypocalcaemia [6]. Intriguingly, high dietary Ca intake restored the decreased expression of the Ca\(^{2+}\) transport proteins independently from 1,25(OH)\(_2\)D\(_3\) [6]. In order to
identify gene products in the kidney that are regulated by high dietary Ca and/or 1,25(OH)2D3, cDNA microarray analysis (15,000 cDNAs) was performed on kidney samples from 1,25(OH)2D3- and high dietary Ca-treated 1α-OHase knockout mice. In this study, 1,25(OH)2D3 induced a significant regulation of ~1000 genes, whereas dietary Ca supplementation of the 1α-OHase knockout mice revealed ~2000 controlled genes as indicated in the Venn diagram (Figure 2) [13]. Interestingly, ~600 transcripts were regulated in both situations, suggesting the involvement in the dietary Ca-mediated rescue mechanism of these vitamin D-deficient mice (Figure 2; for overview data sheets, please see: http://www.genomics.med.uu.nl/pub/bb/kidney/). Conspicuous regulated genes encoded ion channels, channel-interacting proteins, kinases and other signalling molecules, and importantly Ca2+-transporting proteins including the NCX1, calbindin-D28K and the Ca2+ sensor calmodulin. Dietary Ca supplementation in the 1α-OHase knockout mice had a maximum effect on NCX1 expression, suggesting that this basolateral protein is an important extrusion mechanism in the process of transcellular Ca2+ reabsorption. Interestingly, several transcripts, previously not known to be involved in Ca2+ homeostasis, were significantly regulated. An intriguing question is how dietary Ca can regulate gene transcription. First, an increased dietary Ca load might increase the intracellular Ca2+ concentration in Ca2+-transporting kidney cells. Previous studies already indicated Ca2+-responsive elements in the promoter of calbindin-D28K and calmodulin [14]. Second, other reports point to a role for the Ca2+-sensing receptor in the kidney that senses the ambient Ca2+ concentration and transduces signals into the cell at the level of gene transcription (Figure 1) [15,16]. Functional analysis should reveal the regulatory pathways of the Ca2+-sensitive proteins with respect to dietary Ca-mediated rescue of the disturbed Ca2+ balance in vitamin D-deficient animals. The emerging tools of genomics and proteomics are enabling the in-depth study of relationships between diet, genetics and function.
Is vitamin D indispensable?

Several studies supported the notion that vitamin D and Ca supplementation may prevent osteoporotic fractures in people known to be vitamin D deficient [17]. Osteoporosis, a systemic skeletal disease characterized by a low bone mass, is a major public health problem [18]. Nutritional deficiencies have a significant influence on the cause of osteoporosis. Previous studies indicated that a reduced supply of Ca2+ is associated with decreased bone mass and osteoporosis, whereas a chronic and severe vitamin D deficiency leads to osteomalacia, a metabolic bone disease characterized by a decreased mineralization of bone [18]. Results of various clinical trials suggested that Ca2+ supplementation may prevent vertebral fractures in the elderly. As outlined above, Ca supplementation in vitamin D-deficient mice models normalized the hypocalcemia and restored the biomechanical properties of bone [3,4,8]. This treatment, however, does not appear as effective as 1,25(OH)2D3 replacement therapy, since bone growth remained impaired [8]. Hendy and co-workers demonstrated in 1α-OHase and/or VDR knockout mice that optimal dietary Ca absorption requires 1,25(OH)2D3/VDR, whereas skeletal mineralization was dependent on adequate ambient Ca2+ and did not require the 1,25(OH)2D3/VDR system [19]. Together, these studies indicate that Ca2+ cannot entirely substitute vitamin D in mineral and skeletal homeostasis, but the two agents have discrete and complementary functions.

In various clinical conditions associated with a disturbed Ca homeostasis, vitamin D analogues are administered. For instance, the treatment of choice for PDDR and for patients with chronic renal failure is long-term replacement therapy with 1,25(OH)2D3. Notably, the currently applied strategy of vitamin D and Ca2+ supplementation to patients with chronic renal failure has been associated with adverse effects, such as vascular calcification and calciphylaxis. It would be interesting to compare the effectiveness of Ca supplementation with the treatment with vitamin D analogues in these patient groups. Based on present evidence, chelated Ca2+ may be safely and effectively ingested by most people at doses generally recommended for treatment or prevention of Ca2+-related disorders [20]. Further studies on potential dietary Ca-sensitive targets will provide insight into the molecular rescue mechanisms of dietary Ca supplementation.

Acknowledgements. This work was supported by grants from the Dutch Organization of Scientific Research (Zon-Mw 016.006.001) and the Dutch Kidney Foundation (C03.6017).

Conflict of interest statement. None declared.

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