THE ROLE OF DIETARY VITAMIN D AND CALCIUM IN DETERMINING BONE HEALTH AND STRENGTH

A THESIS SUBMITTED IN TOTAL FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT

Adequate dietary vitamin D and calcium intake have shown to be important in regulating skeletal development and bone mineralization. Vitamin D insufficiency is associated with increased fracture risk suggesting that a minimum 25-hydroxyvitamin D (25D) production in bone may be essential for maintaining a healthy skeleton. However, the required level of vitamin D to maintain/improve bone quality is still undetermined. This thesis investigates the regulation of dietary vitamin D on bone in young adult rats as well as the interaction between vitamin D requirement and dietary calcium intake on bone structure and the mechanical measures of bone quality in aged rats. Bone mineral content in trabecular and cortical bones and a number of biochemical factors known to regulate renal metabolism of 1,25D hydroxyvitamin D3 (1,25D) such as PTH, calcium, 25D and 1,25D were examined. Enzymes responsible for the production of 1,25D, 25-hydroxyvitamin D3-1a-hydroxylase (CYP27B1) and the catabolism of 1,25D (25-hydroxyvitamin D-24-hydroxylase (CYP24)) mRNA expression in the kidney was also studied. Furthermore, bone mechanical quality was determined using 3-point bending on rat tibia with the aim to validate mechanical testing as well as determining the effects of varying levels of dietary vitamin D and calcium on bone strength.

We have previously shown that serum 25D levels, which represents the level of vitamin D status is a strong determinant of bone volume. Despite that vitamin D deficiency results in trabecular bone loss in the femur and vertebrae, further cortical bone analysis demonstrated that cortical bone volume, bone mineral distribution and strength was preserved in short term vitamin D deficiency suggesting that the effect of vitamin D deficiency in young adult rats varies between trabecular and cortical regions.

To further understand the reported effects on low dietary calcium induced bone loss, mRNA expressions of the renal enzymes were examined. High renal CYP27B1 mRNA expressions and

serum 1,25D levels in long term low dietary calcium animals suggested that the effects of bone loss may be due to 25D metabolism leading to the reduction in vitamin D status. CYP24 and other liver enzymes were not regulated by the low calcium diet.

We have reported that circulating levels of serum 25D are greater in animals fed a diet containing high levels of calcium. Thus, the effects of a high calcium diet to protect against bone loss may be due to the subsequent effects on the maintenance of circulating 25D levels. μ-CT analysis demonstrated that both femoral and tibial cortical bone volume as well as trabecular bone volume is higher in animals that are fed high calcium and vitamin D diet. Furthermore, 3-point bending demonstrated the greatest maximum load to failure was achieved in the same dietary group. Cortical bone volume and the sagittal loading are both strong determinants of ultimate load suggesting that mechanical forces and bone mineral content are crucial in maintaining the quality and function of bone strength. In addition, these results have validated our mechanical testing suggesting that bone strength is affected despite the subtle changes in cortical bone volume which may be the result of ovariectomy or dietary changes.

The studies of this thesis reveal a complex interaction between dietary calcium and vitamin D, and show that physiological changes in biochemical factors can affect structure and strength in different regions of bone. More importantly, it also demonstrate the optimal levels of dietary calcium and vitamin D that are required to prevent the development of osteoporosis

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to both electronic and this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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