

## EFFECT OF *IN-UTERO* VITAMIN D DEPLETION ON OFFSPRING SKELETAL DEVELOPMENT

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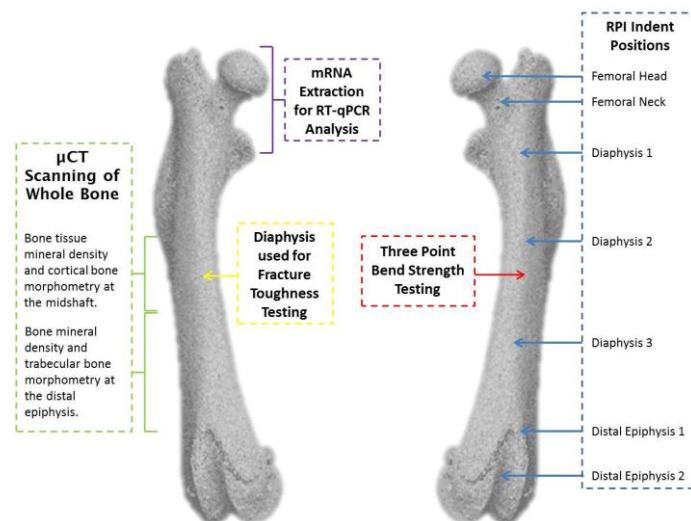
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The impact of vitamin D deficiency during pregnancy on offspring future bone health remains undetermined, despite recognised links between low vitamin D levels and Rickets and osteomalacia in children and adults, respectively. With an estimated one billion people worldwide recorded with deficient vitamin D levels [1], it is important to understand the role of vitamin D in the establishment of bone formation and bone physiology and whether, consequently, this modulates bone quality parameters persistently throughout life.

We postulate that *in-utero* vitamin D deficiency cause changes in bone cell behaviour, thereby affecting the bone quality across the different levels of bone structure. To test this, we have utilised a number of different techniques capable of informing on bone material, structural and mechanical properties (the bone quality framework) across multiple scales.

A rodent model (Sprague-Dawley rats) was used to test the effect of complete vitamin D deficiency *in-utero*. Samples were evaluated at 140 days of age, whereby femora were excised and bone was analysed for osteoblast gene expression, mineral density, micro-morphometry of cortical and trabecular bone, micro-mechanics, fracture toughness and bone strength.



Results were grouped according to gender and control and vitamin D deplete status and measured for statistical significance using student t-tests.

Investigations into osteoblast mRNA expression levels showed that transcript of *Opn* were significantly higher ( $p = 0.02$ ) in the male deplete group when compared to the male control group, whereas *Runx2*, *Coll* and *Ocn* levels did not exhibit significant differences. No significant differences were found in females, where control groups showed higher mean expression levels for all genes evaluated with the exception of *Coll*.  $\mu$ CT analysis of femora showed no statistical differences in bone mineral density between the control and deplete group. Similarly, morphological analysis of cortical and trabecular bone indicated no changes with both genders. Mechanically, no differences were found between the two dietary conditions when comparing i) microindentation at each of the seven femur locations, ii) strength values obtained using three point bending tests and iii) fracture toughness tests.

No consistent and observable differences were found in bone health of 140 day-old animals, despite differences in vitamin D status *in-utero*. Recent studies have shown no links between vitamin D levels in pregnancy and childhood bone health in humans [2], which are supported by these findings. This indicates, at the one time point examined (140 days):

- a) No link is present between bone health and vitamin D depletion or that
- b) Compensatory mechanisms are active to minimise the vitamin D depletion, either during the *in-utero* or developmental phases in this rodent model.

Evidence also exists that alternative means for calcium delivery during pregnancy are present [3], reducing the reliance of the vitamin D pathway in calcium provision to the growing foetus. These results may also be a function of the offspring age investigated, analogous to early adulthood in humans. Thus, any possible changes to risk to chronic diseases, such as osteoporosis are unlikely to be present at this stage. Clearly evaluation at different time points, to identify if recovery from vitamin D deficiency occurs *in-utero* or in early post-natal life and also if changes manifest at a much later phase of life would enhance and inform the current observations and study.

Further longitudinal studies are required to delineate if any compensatory mechanisms are active at the cellular level and whether the effects of vitamin D deficiency can be viewed at other age points. While the current results indicate a limited effect of vitamin D depletion on foetal development, it remains important to determine if there are any critical periods during skeleton maturation where vitamin D status is more highly influential.

## REFERENCES

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