Seaweed Derived Calcium Shows Superior Osteogenic Capabilities Than Calcium Carbonate In An Ovariectomized Rat Model Of Osteoporosis

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Introduction: Osteoporosis degrades bone mass and architecture and results in increased fragility risk. Calcium deficiency has been identified as a major risk factor for osteoporosis although other important minerals in bone health have also been identified including magnesium, copper, manganese, selenium and zinc (1). As a result, calcium supplements, typically calcium carbonate based, are prescribed for postmenopausal women as an aid to prevent osteoporotic fractures. However, these calcium carbonate based supplements have been associated with adverse side effects including increased risk of heart attack and breast cancer. An alternative seaweed-based source of calcium is derived from the skeletal remains of the red marine algae species Lithothamnion Calcareum and is rich in not only calcium but also magnesium and 74 other trace minerals (2). This study used the ovariectomized (OVX) rat, a proven model of bone loss, to examine whether this alternate source of calcium can successfully prevent the loss of bone structure, composition and strength due to osteoporosis.

Methods: ***In vivo study:*** Eighty-eight female, retired breeder Wistar rats, both OVX and age matched controls (Harlan Laboratories,UK) were allowed to acclimatize for 5 days before the start of the experiment (Week 0). The experiment was approved by the Animal Ethics Committee of Trinity College Dublin, Ireland. Two diets were used in this study, a standard rat chow (RM1) containing calcium carbonate ($CaCO_3$) and RM1 minus $CaCO_3$ which was supplemented with Aquamin, a seaweed derived calcium (SDS, UK). For the seaweed supplemented feed, Aquamin was added at a concentration such that calcium levels were equal in both feeds. The rats were divided into four groups: (1) control (n=28; RM1), (2) OVX plus $CaCO_3$ (n=24, RM1), (3) OVX plus Seaweed Supplement (n=24) and (4) OVX plus Seaweed Supplement Delay (n=12; RM1 followed by seaweed supplemented RM1 after week 8). Animals were allowed to eat and drink ad libitum and euthanized at weeks 0, 2, 8, 12 and 20. *Micro Computed Tomography (MicroCT):* Following sacrifice the proximal region of the left tibia was scanned (µCT40, Scanco, Switzerland). Microarchitecture was analyzed at a resolution of 8µm, at 70kVp and 114µA and a threshold value of 210 was selected. *Fourier Transform Infrared Spectroscopy (FTIR):* Measurements of bone composition were carried out on samples from the proximal tibia using a Perkin Elmer Spotlight 400N FTIR imaging system and images were acquired in reflection imaging modes between 600 and 4000cm^{-1}. Background measurements were acquired on a barium fluoride window with 120 scans per pixel whereas 8 scans per pixel were recorded from the sample. *Mechanical Testing:* A Nano indenter XP system (MTS System Corporation, Oak Ridge,TN) was used to measure tissue level mechanical properties of proximal tibia sections. The Young's Modulus and contact hardness were assessed using an AccuTip™ diamond Berkovich indenter tip. *Statistics:* Test data were analyzed using ANOVA (SigmaStat, San Jose, Ca) and a paired Student's t-test was performed between each measurement.

Results: The OVX + $CaCO_3$ group showed large changes in structural parameters indicating the development of ovariectomy induced bone loss (Fig. 1 A and B). Significant reductions were observed in bone volume fraction at week 8 while trabecular number was reduced and trabecular separation was increased by week 12. In contrast, animals receiving the seaweed supplement showed a slower rate of bone loss with no significant changes in trabecular parameters compared to the controls. By week 20 there was significant preservation of bone volume fraction in the seaweed supplemented group relative to OVX + $CaCO_3$. The seaweed supplement delay group showed strong trends although no significant improvement in trabecular

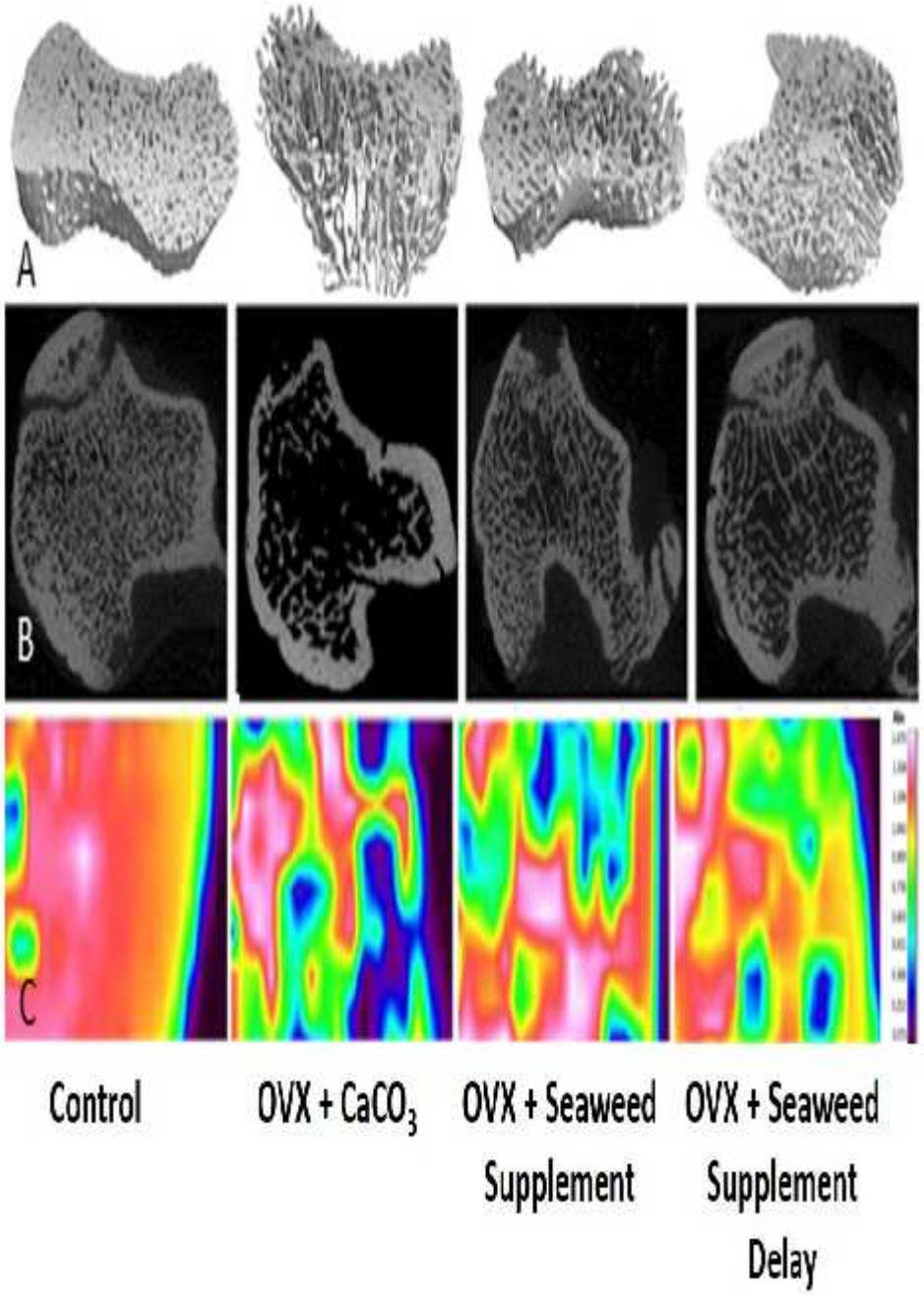

Figure 1: Week 20 (A) 3D microCT reconstruction of the proximal tibia (B) microCT slice of the proximal tibia (C) infrared absorbance spectra where red indicates increased absorbance indicating increased mineralization.

architecture was measured. Qualitative evaluation of the FTIR images at week 20 revealed increased absorbance (red) (Fig. 1C) indicative of increased mineralization in the control samples relative to the OVX + $CaCO_3$ group. This was confirmed by quantifying the images which found a 50%

reduction in the mineral/matrix ratio in the OVX + $CaCO_3$ group relative to the controls ($p<0.05$). In contrast in the OVX + seaweed supplement group, mineral/matrix ratio was increased almost 2-fold relative to OVX + $CaCO_3$ ($p<0.05$). No significant difference was measured between control, OVX + seaweed supplement and OVX + seaweed supplement delay. By week 20, estrogen deficiency due to ovariectomy reduced the modulus of trabeculae from the proximal tibia relative to controls ($p<0.05$). Immediate seaweed supplementation preserved both the modulus and hardness in trabecular bone relative to OVX + $CaCO_3$ ($p<0.05$).

Discussion: In the current study both the regular chow, containing calcium carbonate, and the seaweed supplemented chow have equal amounts of calcium. The significant preservation of the trabecular structure, composition and strength in the seaweed supplemented group is therefore brought about by the unique form in which the calcium is supplied and the fact that the supplement also contains 75 additional minerals including magnesium, manganese, selenium and zinc. From microCT analyses, although only bone volume fraction was significantly altered by seaweed supplementation it is important to note that trabecular number, separation and thickness are all incorporated in the bone volume fraction measurement. In addition to preserving the structure, seaweed supplementation ensured that the composition of the bone remained unchanged and this in turn conserved the material properties of the bone. A significant factor to remember is that this seaweed supplement is a neutraceutical and not as potent as pharmaceuticals, therefore to achieve a significant preservation in trabecular bone after only 20 weeks is a major outcome. Crucially this study has shown that this seaweed supplement offers superior osteogenic potential to that of calcium carbonate.

Significance: This study has shown that oral ingestion of a seaweed derived calcium supplement has the ability to preserve trabecular bone structure, composition and strength in the proximal tibia of rats following ovariectomy better than calcium carbonate. Thus there may be potential for the use of these seaweed based calcium supplements rather than calcium carbonate based to aid in the prevention of bone loss in post-menopausal women.

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