

EFFECT OF DIFFERENT DOSES OF VITAMIN D₃ SUPPLEMENTATION ON MANDIBULAR BONE IN RATS

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ABSTRACT

INTRODUCTION: vitamin D is one of the most important biological regulators of calcium metabolism which ultimately promotes healthy bones. It is also helpful in reducing inflammation as well as enhancing immune functions and cell growth. Despite its importance, its deficiency is prevalent worldwide. Moreover, it is difficult to obtain this vitamin from diet because it is not present in many foods. Consequently, Vitamin D₃ (cholecalciferol) supplementation is nowadays used worldwide to maintain optimal bone health. However, studies evaluating the effect of standard doses of vitamin D supplements on bone have been inconsistent. In addition, the effects of higher doses have not been revealed and needs further studies.

AIM OF THE STUDY: to determine the effect of supplementation of varying doses of dietary vitamin D₃ on the structure of mandibular bone in albino rats.

MATERIALS AND METHODS: Thirty adult female rats were randomly divided into three groups: **Group I** control, while **Group II** and **Group III** received daily dose of vitamin D₃ (400 IU/Kg) and (1000 IU/Kg) respectively. All rats were fed on same diet. After 6 weeks animals were euthanized and their mandibles were dissected out. The effects of each dose of vitamin D₃ on the mandibular bone structure were assessed by scanning electron microscope and energy dispersive X-ray microanalysis.

RESULTS: After 6 weeks, Group I control and Group II supplemented with 400 IU/day, showed loss of its normal cortical bone smoothness. Moreover, the trabecular bone showed wide bone marrow spaces with thin trabeculae. However, group III supplemented with 1000 IU/day, showed smoother and more uniform cortical bone surface with wider trabeculae and narrower marrow spaces compared to the other groups.

CONCLUSIONS: This study showed that administration of vitamin D₃ (1000 IU/day) enhanced bone architecture in adult rats. However, the administration of 400 IU/day showed slight enhancement in bone structure.

KEYWORDS: vitamin D₃, Cholecalciferol, Bone architecture, scanning electron microscope.

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INTRODUCTION

Vitamin D₃, a fat soluble vitamin, is responsible for maintenance of calcium homeostasis along with the two peptide hormones calcitonin and parathyroid hormone. It promotes calcium absorption from the gut thus keeping adequate levels of serum calcium and phosphate to keep normal bone mineralization and to prevent hypocalcemia. In addition it is important for the process of bone growth and bone remodeling by osteoblasts and osteoclasts (1,2).

It is found in very few food sources including oily fish such as salmon, sardines, mackerel and some fish oils as cod liver oil. In addition to some foods including milk, some cereals, orange juice, as well as, some yogurts which are fortified by variable amount and forms of vitamin D in many countries. It is also found as a dietary supplement. Moreover, it is synthesized by the body when the skin is exposed to ultraviolet rays of the sun (1,3,4).

Vitamin D obtained from all these sources is biologically inert and must undergo two hydroxylations in the body for activation. The first occurs in the liver and converts vitamin D to 25-hydroxyvitamin D [25(OH) D], also known as calcidiol. The second occurs primarily in the kidney and forms the physiologically active hormone 1 α ,25-dihydroxyvitamin D [1 α ,25(OH)₂D], also known as calcitriol (5).

Nutrition experts and scientists believe that about two-thirds of the world is not getting enough vitamin D to maintain bone health that lower their risks for fractures and improve tooth attachment. This vitamin D insufficiency is also believed to increase risks for cancers (6).

Recently, the prevalence of vitamin D deficiency has increased and rickets has spread in many developing countries as a public health problem. Moreover babies and toddlers who have been breast-fed without receiving vitamin supplements during this period, or whose mothers

did not get vitamin D supplements during pregnancy had shown deficiency of the vitamin. This deficiency is also a significant risk factor for the development of osteoporosis later in old age (7).

Currently the recommended daily intake of vitamin D3 in the USA is 400–800 IU of vitamin D3 (8). Oral vitamin D supplementation between 700 to 800 IU/day appears to reduce the incidence of hip and any non-vertebral fractures in elderly persons (9).

Higher doses of vitamin D above the recommended level have been questioned whether it further improves bone health. Many experts believe that these recommended doses are too low as studies have shown that more vitamin D3 than the recommended guidelines needs to be consumed to reach more health benefits (8,10,11). Researches also revealed that consuming 1,000 IU daily would help 50% of people to reach a vitamin D blood level of 33 nanograms per milliliter (12–14)

Bone status of the jaws is an important issue since the decrease in alveolar bone density is a prominent cause for periodontitis and tooth loss. It is also important to know the bone quantity and bone quality of the jaws when planning prosthetic and pre-prosthetic surgical treatment. Systemic loss of bone density in osteoporosis, including that of the oral cavity, may provide a host system that is susceptible to destruction of periodontal tissue and bone fractures (15,16).

Studies examining the effect of standard doses of vitamin D on bone health, especially the jaw bones, have been inconsistent (17–19). In addition, the effects of higher doses of the vitamin are not well known and needs further studies especially at the ultrastructural level of investigation. Thus this is why this study was carried out. However, the null hypothesis of this study assumes that no differences will be found in alveolar bone structure of rats taking different doses of vitamin D3 supplements otherwise, the results will prove the opposite.

MATERIALS AND METHODS

The study design was approved by the Ethical Committee of Faculty of Dentistry Alexandria University. Thirty adult female albino rats weighing 200-250 grams approximately 5-6 months of age were used in this study. These animals were obtained from the Institute of Medical Research, Alexandria University.

The rats were housed in covered cages to limit exposure to sunlight (20). They were supplied with a regular adjusted diet throughout the whole experimental period which lasted for 6 weeks.

The animals were randomly divided into three equal groups (10 in each group) as follows:

Group I (control group) were fed on normal balanced diet without vitamin D3 supplemented doses.

Group II (400 IU/Kg) were fed on normal balanced diet with vitamin D3 supplemented dose of 400 IU/Kg daily.

Group III (1000 IU/Kg) were fed on normal balanced diet with vitamin D3 supplemented dose of 1000 IU/Kg daily. Vidrop (manufactured by Medical Union Pharmaceuticals, Egypt) supplied in the form of liquid was used as a

supplemented vitamin D3. Each drop contains 100 IU of vitamin D3 (cholecalciferol) (equivalent to 0.036 ml of the drug). All the rats were given 1 ml pure olive oil daily by intraoral administration.

The body weights of rats were recorded weekly throughout the experimental period (successive 6 weeks) to determine the dose of the supplemented vitamin D3.

The doses of the vitamin D3 supplemented groups (II, and III) were given per average kilogram of body weight which was approximately 250 grams. The volume of solution given orally was calculated according to the weight of each rat in each group.

By the end of 6th week, all rats were euthanized. The mandible of each rat was dissected out and prepared for Scanning Electron Microscopic examination (SEM) and Energy Dispersive X-ray microanalysis (EDX).

Statistical Analysis

Statistical analysis of the calcium and phosphorus concentrations was done using IBM SPSS software Package version 20.0. Post Hoc test was used for comparing the three studied groups for normally distributed quantitative variables (21).

RESULTS

1-Scanning electron microscope results

Group I (control group)

The buccal cortical plates' surfaces exhibited smooth surface areas with adjacent irregular areas and irregularly outlined borders of nutritive canal (Fig. 1). The exposed surface of the trabecular bone showed thinning in its trabeculae with wide marrow spaces encircled with areas showing normal thickness and narrow marrow spaces (Fig. 2).

Group II (supplemented with vitamin D3 400 IU/Kg daily)

The buccal cortical plates showed shallow irregular surface topography adjacent to smooth areas with regular outline of the nutrient canals (Fig.3). The trabecular bone exhibited wide bone marrow spaces with reduction in the thickness of bone trabeculae in between narrow marrow spaces and thick trabeculae (Fig.4).

Group III (supplemented with vitamin D3 1000 IU/kg daily)

The surfaces of the buccal cortical plates of the bone showed a generalized improvement than group II. Smoother pattern and more uniform surface of the plates were observed than in the previous two groups. Moreover the nutrient canals appeared narrower and with regular openings (Fig.5). The trabecular bone showed smaller and shallower marrow spaces with thicker bone trabeculae (Fig.6).

2- Energy Dispersive X-ray microanalysis (EDX)

The calcium and phosphorous levels in different groups are summarized by means and standard deviation in table (1).

There was statistically insignificant increase in calcium level and decrease in phosphorous level in group II (supplemented by 400 IU/day) in relation to control group I (P1 for calcium=0.564) and (P1 for phosphorous=0.066). In group III (supplemented by 1000 IU/day), there

was a significant increase in calcium level and decrease in phosphorous level in relation to control group I (P2 for calcium=0.001) and (P2 for phosphorous <0.001). Moreover, the difference in calcium and phosphorous levels between group II and group III was statistically significant (P3 for calcium=0.008) and (P3 for phosphorous=0.007).



Fig. 1: Scanning electron micrograph (SEM), group I control, showing the buccal cortical plate of bone with smooth surface areas adjacent to other areas with irregular surface (white arrows). Note the irregularly shaped outline of the nutritive canal (black arrow). (X500)

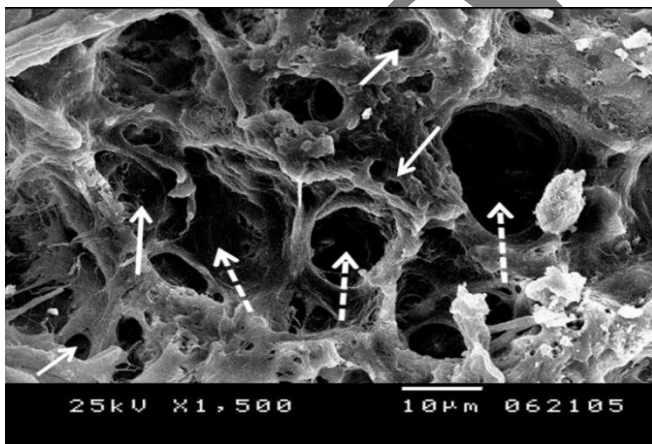


Fig. 2: SEM, group I control, of the exposed trabecular bone showing normal thickness of some bone trabeculae with narrow marrow spaces (arrows), while others appear thin with wide marrow spaces (dotted arrows). (X1500)

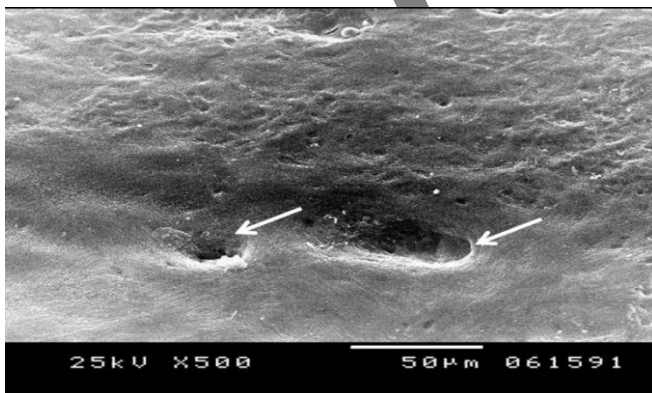


Fig. 3: SEM, group II supplemented with 400 IU/day, showing the buccal cortical plate with areas of irregularities on the surface adjacent to normal smooth surface. Note the regular and narrower outline of the nutritive canal (arrow). (X500)

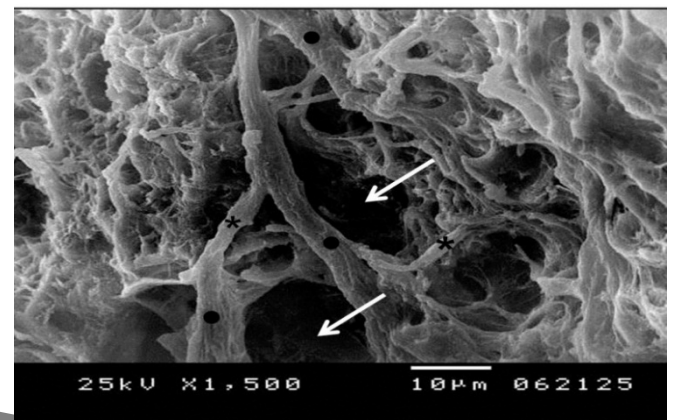


Fig. 4: SEM, group II supplemented with 400 IU/day, showing appearance of thin bone trabeculae (asterisks) with wide marrow spaces (arrows). Note also the presence of thick bone trabeculae (dots). (X1500)

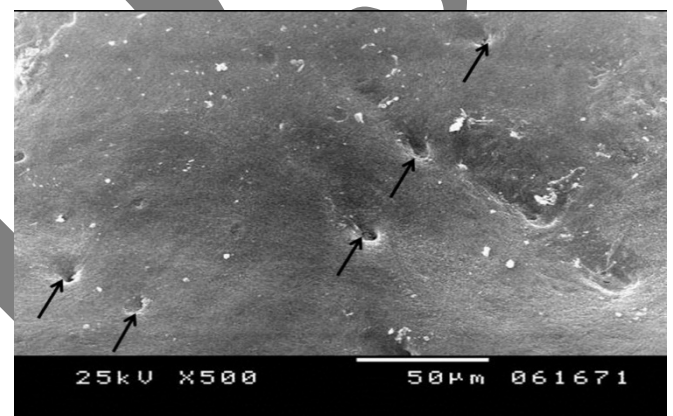


Fig. 5: SEM, group III supplemented with 1000 IU/day, showing nearly smooth and uniform buccal cortical plate with normal outline of the nutrient canals (arrows). (X500)

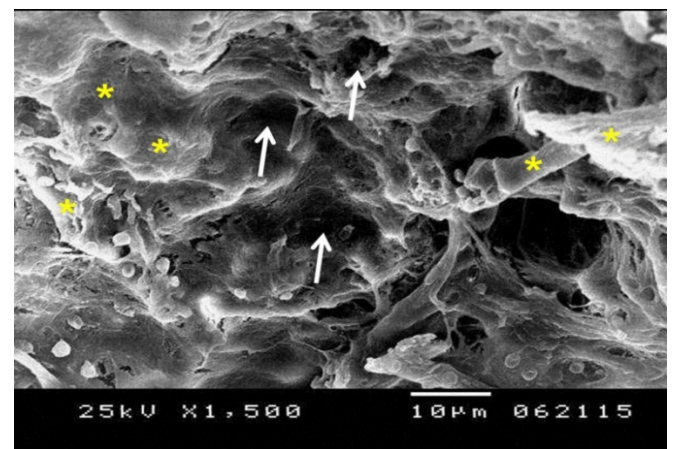


Fig. 6: SEM, group III supplemented with 1000 IU/day, of the exposed trabecular bone having smaller and shallower

bone marrow spaces (arrows) with broader bone trabeculae (asterisks) than the previous groups. (X1500)

Table (1): Comparison between the different studied groups according to calcium and phosphorus

	Group I	Group II	Group III	p
Calcium				
Min. – Max.	64.80– 66.50	63.60– 70.30	67.60– 73.80	0.001*
Mean ± SD.	65.52±0.64	66.72 ± 2.64	70.82±2.15	
Median	65.45	66.35	70.50	
Sig. bet. grps	p ₁ =0.564,p ₂ =0.001*,p ₃ =0.008*			
Phosphorus				
Min. – Max.	33.50– 36.20	29.70– 33.40	26.20– 32.40	<0.001*
Mean ± SD.	34.65±0.93	32.43 ± 1.38	29.18±2.15	
Median	34.55	32.90	29.50	
Sig. bet. grps	p ₁ =0.066,p ₂ <0.001*,p ₃ =0.007*			
Calcium/ Phosphorus ratio				
Min. – Max.	1.79–1.99	1.90 2.37	2.09–2.82	<0.001*
Mean ± SD.	1.89±0.07	2.06 ± 0.16	2.44±0.25	
Median	1.89	2.04	2.39	
Sig. bet. grps	p ₁ =0.258,p ₂ <0.001*,p ₃ =0.006*			

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the different studied groups

p1: p value for comparing between Group I and Group II

p2: p value for comparing between Group I and Group III

p3: p value for comparing between Group II and Group III

*: Statistically significant at $p \leq 0.05$

DISCUSSION

Vitamin D deficiency is a prevalent medical condition that affects bone health leading to bone growth retardation and development of classic signs and symptoms of rickets, osteopenia and osteoporosis (22).

There had been a debate considering the use of vitamin D as a treatment for adulthood osteoporosis as it has been reported that vitamin D supplementation in adulthood reduces (9,23), increases (8,24), or may even cause no effect on incidence of fractures due to osteoporosis (25).

There are two main types of vitamin D. The first is vitamin D3 (cholecalciferol), which is found in animals including fish and it is also made by the skin when exposed to sunlight. The second is vitamin D2, which comes from plant-based foods including mushrooms. In this study we used vitamin D3 because studies revealed that vitamin D3 is more effective than vitamin D2 (26,27).

Regarding the doses of vitamin D3 supplements used in the present study, we chose to use two different daily doses of vitamin D3 as follows : Low dose (400 IU) recommended by the study of Sanders et al (8) and another higher dose (1000 IU) suggested by some studies to be effective in reaching blood level of 33 nanogram per milliliter of serum 25(OH)D which is considered as a sufficient level (12,13).

Regarding the use of olive oil as a vehicle substance for the vitamin D3 supplement in our study, It was shown that vitamin D in an oil vehicle produces a greater bioavailability than if used alone (28,29).

In the present study, the enhancement of bone mass occurred in rats supplemented by vitamin D especially the highest level of vitamin D3 (1000 IU). This is consistent with a previous study by Lee et al (30), where vitamin D interventions resulted in greater effects on trabecular bone.

A systematic meta-analysis also confirmed that the use of vitamin D supplementations higher than 400 IU, provide a preventive measure against fractures in elderly people. It stated that an oral vitamin D dose of 400 IU/day was not sufficient for fracture prevention. These results agree with our observations in lower doses of the vitamin (400 IU), where slight enhancements were seen in bone compared to the control group (9).

In another meta-analysis targeting people aged 50 years or older a significant difference was obtained between the effects of different vitamin D3 doses. It also recommended the use of a minimum dose of 800 IU of the vitamin (23).

The Scanning electron microscopic results showed an enhancement in bone architecture in the 1000 IU vitamin D3 supplemented group. This is coincident with the study of Eisman et al (31) who confirmed the direct relation between vitamin D and osteoblast action in bone formation where a decrease in osteoblastic matrix formation was found in vitamin D deficient rats.

On the other hand, Smith et al (24) found that an annual intramuscular injection of vitamin D did not reduce fracture risk when compared with placebo. This finding is consistent with a previous study where annual single high-dose of vitamin D3 (500 000 IU) administered orally to older women resulted in an increased risk of osteoporotic fractures, suggesting the necessity for further studies concerning the safety of high-dose vitamin D supplementation (8).

The study of Michaelsson et al (25) postulated that vitamin D3 might not have a protective effect on bone, where it showed no evidence that a high dietary calcium or vitamin D intake plays a role in the primary prevention of osteoporotic fractures in women aged 40–74 years.

An alternative strategy for preventing osteoporosis in old age is to optimize bone mass during growth through giving vitamin D3 supplementations during adolescence. A number of interventional studies examining the effects of vitamin D3 supplementations in adolescents have reported significant increases in bone mineral content (32,33). This increase in bone mineral content supports our elemental microanalysis results which revealed that there is a

significant increase in calcium concentration in relation to phosphorus in the group given the highest vitamin D3 supplement (1000 IU) in comparison to the other two groups; the control and the group given 400 IU of the vitamin. These results were also supported by Matsumoto et al (34) who revealed that 1,25-dihydroxyvitamin D3 supplementation caused an increase in lumbar and hip bone mineral density in individuals with osteoporosis.

A recent study in 2017 supported the use of vitamin D3 supplementation, during adolescence, for achieving a higher peak bone mass in adulthood and thereby preventing osteoporosis. The study suggested using high levels of vitamin D3 supplementations, reaching to 20,000 IU/kg during early adulthood leading to increase in bone health by improving bone material strength (14).

However other systematic reviews done to reveal the effectiveness of vitamin D supplementation for improving bone mineral density in children and adolescents and to reveal if effects vary with factors such as vitamin D dose. It was concluded that no significant effect on bone mass density neither on bone mineral content (10,35).

CONCLUSIONS

The findings of this study provide evidence that vitamin D3 supplementation with 1000 IU/day for 6 weeks provide better bone architecture than the low dose 400 IU/day which showed slight enhancement in bone structure. It also showed a significant increase in calcium concentrations in bone. Thus it is recommended to use the higher dose of vitamin D3, especially in old aged people.

Conflict of interest

We declare that we have no conflicts of interest.

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