 DOI: **10.21608/adjalexu.2022.123431.1257**

EFFECT OF HYPERVITAMINOSIS A ON GROWTH OF MANDIBULAR CONDYLE IN RATS

Shimaa A. Halawa [1*] BDS, Shadia H. Abdel majeed [2] PhD, Mounir M. El –Adawy [3] PhD, Hagar S.Abdel Fattah [4] PhD

ABSTRACT

BACKGROUND: Vitamin A is present in our food such as meat, dairy products, and vegetables. A control balanced diet maintains our nutritional needs of vitamin A. Over consumption of products as well as supplementations with vitamin A increases the risk of hypervitaminosis A. Hypervitaminosis A has been associated with thinning of the long bones and reducing of bone formation

OBJECTIVES: to investigate the effect of over as well as optimum doses of vitamin A on growth of mandibular condyle in Wister albino rats.

MATERIALS AND METHODS: Eight healthy (2month old age) male Wister albino rats weighting (274 - 375grams) were used in present study. The rats were randomly divided into 2 equal group, study and control group .In the control group; rats fed a standard diet containing an optimum dose of vitamin A (12 IU/g of each pellet). In the study group, rats fed a standard diet supplemented with overdose of vitaminA (1700 IU /g of each pellet). After 2months, all rats were euthanized. Blood samples were collected from all rats the day after administering the last dose of vitamin and the concentration of vitamin A in serum was determined for all samples. The condyles of each rat of all groups were dissected out and processed for histological study and histomorphometric analysis.

RESULTS: In the study group, the histological examination revealed marked reduction in thickness of hypertrophic zones and increase in the width of chondroblastic zones of condylar cartilage. Bone trabeculae were thin with irregular outline. The subchondral bone showed marked bone resorption with numerous osteoclasts in Howship's lacunae as compared with the control group.

CONCLUSIONS: findings from the present study have indicated that, hypervitaminosis A increases resorption of the bone and diminishes endochondral bone growth.

KEY WORDS: Hypervitaminosis A, mandibular condyle growth, albino rat.

---

1. Demonstrator of Oral Biology, Faculty of Dentistry, Alexandria University.
2. Professor of Oral Biology, Faculty of Dentistry, Alexandria University.
3. Professor of Animal Production, Animal Production Department, Faculty of Agriculture Alexandria University.
4. Lecturer of Oral Biology, Faculty of Dentistry Alexandria University

*** *Corresponding Author*:**
shimaa.halawa.dent@alexu.edu.eg

INTRODUCTION

Vitamin A is fat soluble compounds. There are two types of vitamin A, the first type is retinol, retinal and retinoic acid (derivative of animal source) and the second type is carotenoid (derivative of plant source) (1-4).

The retinoid form is found in eggs, liver, milk and fortified cereals (5) , while Carotenoids are found in carrots, collards, spinach, and squash (1, 2).

Vitamin A is an essential nutrient needed in small amounts for the normal function of the visual system, development and growth, immune function, maintenance of epithelial cellular integrity and reproductive system (6).

The absorption of vitamin A occurs in the intestine with the help of lipids .It is stored mainly in the liver, kidney, and adipose tissues (7).

Severe deficiency of vitamin A is known to produce corneal Xerophthalmia, blindness and keratomalacia in children (6).Over dose of vitamin A leads to vision problems, changes in the skin, and bone pain. Chronic cases of hypervitaminosis A may result in liver damage and pressure on the brain (8).

Vitamin A supplementations represent the retinoid form of vitamin A (9). The retinoid type is efficiently absorbed at rates of 70 –90%, while the carotenoids type are absorbed much less efficiently at rates of 20 -50%. The retinoid type of vitamin A is more liable to cause vitamin A toxicity than carotenoids type (10). Excessive dietary intake

of beta- carotene can lead to carotenodermia. Carotenodermia is a condition that is characterized by orange-yellow discoloration of the skin (11, 12).

Experimental studies on rodents have shown that a high intake of vitamin A leads to thinning of the long bones, reduction of bone formation (13) and induction of spontaneous bone fracture in animals(14).

In human, increased intake of vitamin A has been associated with increased bone fragility and fracture risk(15).

The mandibular condyle is a center of growth from birth until adulthood in human (16). The mandibular condylar cartilage provides regional adaptive growth, movable articulation and endochondral bone growth (17).

Several studies showed the effect of vitamin A on the skeletal bone; however no available studies show the effect of vitamin A on growth of cartilaginous mandibular condyle. So, the aim of this study is to evaluate the effect of over as well as optimum dose of vitamin A on growth of mandibular condyle in Wister albino rats.

The study was conducted with the null hypothesis that there is no effect of over dose of vitamin A on growth of mandibular condyle in Wister albino rats.

MATERIAL AND METHODS

Study sample

Eight healthy (2month old age) male albino Wister rats (weighting 274 - 375 grams) were used in this study (18). Animals were obtained from the animal house of Medical Research Institute, Alexandria University. Rats were housed in specially designed wire mesh bottom cages. The animals were supplied a regular diet and free access to water during the whole experimental period (19). All animal procedures followed the National Research Council guidelines for the care and use of laboratory animals (20). The study was performed after gaining the approval of the Research of Animal Ethics Committee, Faculty of Dentistry, Alexandria University. Number of ethical committee is 0159 and IORG is 0008839.

Grouping

Eight Rats (2month old age) were randomly divided into 2 groups:

Group I (control group): 4 rats were fed a standard diet containing an optimum dose of vitamin A (12 IU /g of each pellet).

Group II (study group): 4 rats were fed a standard diet supplemented with overdose of vitamin A (1700 IU /g of each pellet).

Vitamin A was added to the rat pelleted diet in the form of retinyl palmitate(A-Viton). The content of each capsule was in the form of oil soluble material. It was dissolved in vegetable oil and then sprayed on diet on which each gram of pelleted diet containing (12 IU) as optimum dose of vitamin A and (1700 IU) as over dose of vitamin A.

Euthanization time

All rats were euthanized by overdose of diethyl ether after 2 months. The remains of rats' bodies were rid of by burial.

Histological study:

the mandibular condyles of each rat were dissected out and were fixed in10% neutral buffered formalin for 24h .After fixation, specimens were decalcified in 10% trichloro-acetic acid, washed in tap water over night and then dehydrated in ascending concentrations of alcohol, cleared in xylene and then infiltrated and embedded in low melting point (56^{o}C) paraffin wax (21).Tissue blocks were cut coronal at a 5 µm thickness with a microtome (22).Sections were stained with Hematoxylin and Eosin stain (H&E) for general examination (21) .

Quantitative determination of serum vitamin A concentrations: (7, 23)

At the end of the experimental period and before euthanization, blood samples were collected from the orbital plexus of rats. This technique is called "orbital venous plexus bleeding". The concentration of vitamin A in serum was determined for all samples using rat vitamin A ELISA kit.

Histomorphometric analysis:

Histormorphometric analysis was done using image J software(24) to measure the percentage of bone surface area of the condyle ,the thickness of chondroblastic and hypertrophic zones.

Statistical analysis: Data obtained from biochemical analysis and histomorphometric analysis were fed to the computer using SPSS software package version 23. Normality was checked using Shapiro Wilk test, box plots and descriptive data was not normally distributed and presented using mainly Median, Inter Quartile Range (IQR) and Minimum and Maximum values in addition to Mean, Standard deviation (SD). Groups are compared regarding concentration of vitamin A, bone surface area of the condyle, the chondroblastic and hypertrophic zones thickness using Mann Whitney U test. Significance level was set at *P* value of 0.05. All tests were two tailed.

RESULTS

I) Histological results

Group I (control group)

In this group, the condyle showed normal thickness of cartilaginous zones: the fibrocartilage zone, proliferative zone, chondroblastic zone and hypertrophic zone (fig.1). The chondroblastic zone showed numerous mature chondrocytes with open faced nucleus and wide intercellular spaces. The hypertrophic zone showed larger chondrocyte with signs of degeneration and disappearance of nucleus. The hypertrophic zone was followed by zone of erosion with proliferative blood vessels and numerous chondroclasts resorbed the mineralized matrix (fig.2a). The osteogenic zone showed well-developed bone trabeculae surrounding normal sized, cellular bone marrow spaces (fig.2b).The bone trabeculae of the condyle showed fan shaped appearance (fig.1). The endosteal surface of bone showed a regular outline lined by continuous layer of plump to flattened osteoblasts (fig.2b).

Group II (study group):

The condylar cartilage revealed a thin hypertrophic zones with signs of degeneration and complete disappearance of chondrocytes (fig.3, 4a, 5,6a) and wide chondroblastic zones with reduction of intercellular spaces in comparison to control group (fig5, 6a).the erosive zone revealed numerous chondroclasts than control group with proliferative blood vessels(fig.6b) .The osteogenic zone revealed thin bone trabeculae with irregular outline lined by numerous osteoclasts in Howship's lacunae and wide bone marrow (fig.3,4b,5,6b). Areas of woven bone had been detected in the osteogenic zone. (fig.3, 4b, 5,6b).

II) Histomorphometric analysis:

A) Bone surface area

The percentages of the bone surface area was greater in control group as compared to study group and it was statistically significant (p=0.01) table (1).

B) Thickness of chondroblastic zone of the condyle

Thickness of chondroblastic zone showed no statistically significant difference (p=0.19) between control and study group despite the mean value of thickness were higher in the study in comparison to control group table 2.

C) Thickness of hypertrophic layer of the condyle

The hypertrophic layer of the condyle showed reduction in thickness in study group as compared to control group and it was statistically significant (p=0.027) table (3).

III) Biochemical analysis

The mean value of serum vitamin A concentration was greater in study group as compared to control group and it was statistically significant table (4) (p= 0.03) .

Figure (1): Light photomicrograph (LM) (control group, 4months) showing normal thickness of fibrocartilage, proliferating, chondroblastic and hypertrophic zones. Well-formed bone trabeculae with normal size of bone marrow. C: cartilage BM: bone marrow B: bone H&E, stain (a) x40, (b) x100.

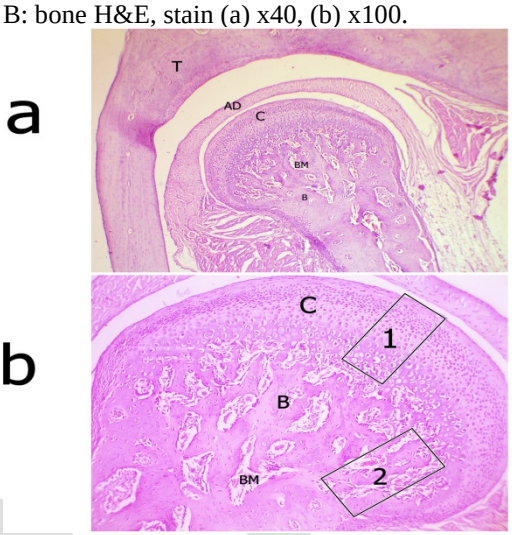

Figure (2): Higher magnification of insets in the previous figure showing a: inset 1 normal thickness of cartilaginous zones F: fibrocartilage zone, P: proliferative zone, C: chondroblastic zone, H: hypertrophic zone. Numerous chondroclasts in erosive zone (arrow) b: inset 2 thick bone trabeculae and plumb to flat osteoblast cell lining the endosteal bone surface (arrow). H&E, stain x400.

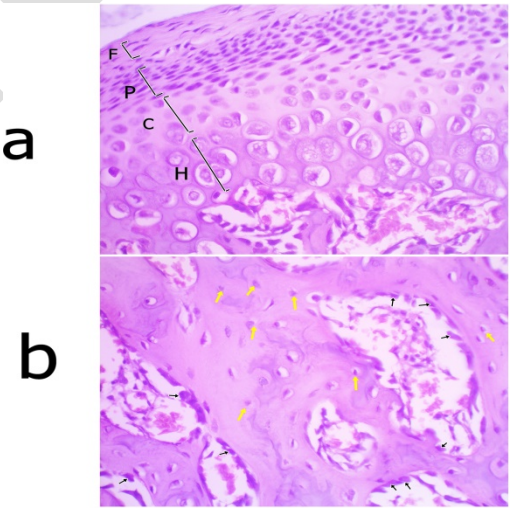

Figure (3): Light photomicrograph (LM) (study group, 4 months) showing reduction in thickness of hypertrophic zone as compared to control group. Thin bone trabeculae with wide bone marrow spaces. Note the islands of woven bones (star) C: cartilage BM: bone marrow B: bone T: temporal bone H&E, (a) x40, (b) x100.

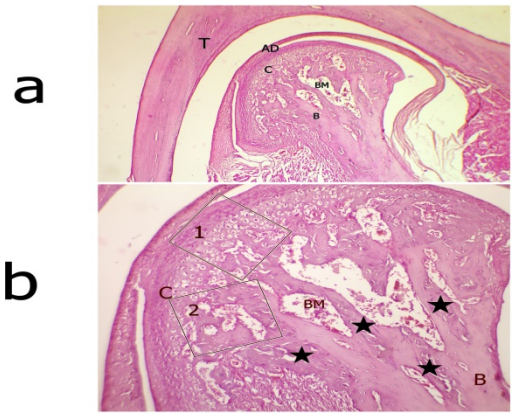

Figure (4): Higher magnification of the insets in the previous figure a: inset 1 showing thinning of the hypertrophic zone as compared to control group with pyknotic or complete disappearance of the chondrocyte nuclei (arrow), numerous chondroclasts in erosive zone (dotted arrow) F: fibrocartilage zone, P: proliferative zone, C: chondroblastic zone, H: hypertrophic zone. .b: inset 2 showing irregular bone surface studded with osteoclasts in Howship's lacunae (arrow). Note the islands of woven bones (star) .H&E stain x400.

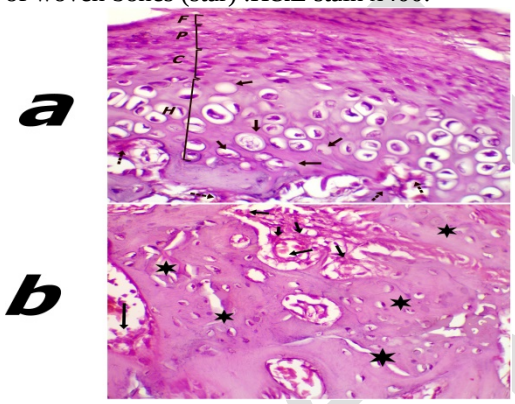

Figure (5): Light photomicrograph (LM) (study group, 4months) showing thinning of hypertrophic zone and widening of the chondroblastic zone as compared to control group with thin bone trabeculae and wide bone marrow spaces.). Note the islands of woven bones (star). C: cartilage BM: bone marrow B: bone T: temporal bone H&Ex100.

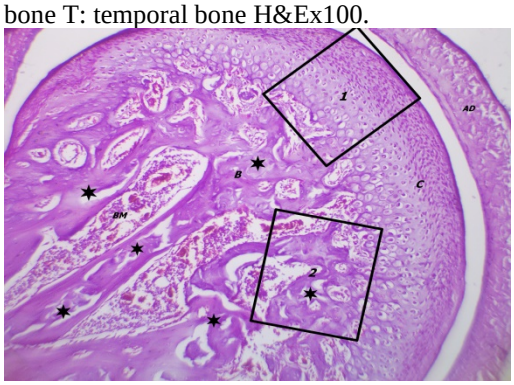

Figure (6): Higher magnification of the insets in the previous figure a: inset 1 showing reduction in the thickness of hypertrophic zone and increase in thickness of chondroblastic zone as compared to control group. Chondrocyte lacunae with sign of degeneration (arrow), F: fibrocartilage zone, P: proliferative zone, C: chondroblastic zone, H: hypertrophic zone. b: inset 2 showing irregular bone surface studded with osteoclasts in Howship's lacunae (dotted arrow). Note the areas of woven bone (star) and numerous chondroclasts in erosive zone (arrow). H&E stain x400.

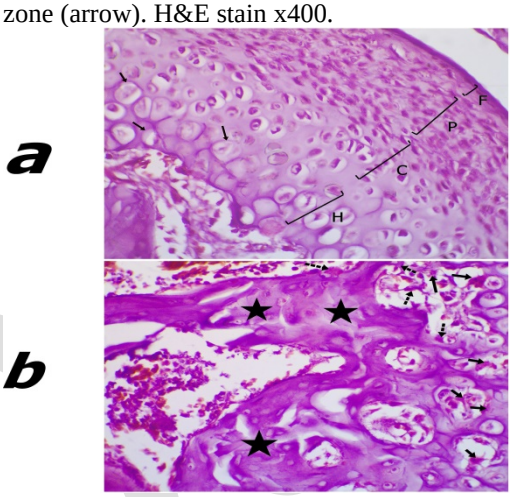

Table (1): Comparison between the study and control groups regarding the bone surface area of the condyle

		Control Group	Study Group	Test (P value)
Percent (%)	**Mean (SD)**	65.13(9.78)	52.14(8.70)	0.01*
	Median (IQR)	69.14(10.98)	49.05(6.52)	
	Min - Max	48.7575.59 -	42.2168.79 -	

*Statistically significant difference at *p* value≤0.05

Table 2: Comparison between the study and control groups regarding the thickness of chondroblastic zone:

	Control Group	Study Group	Test (P value)
Mean (SD)	0.0268(0.01)	0.0356(0.02)	(0.19*)
Median (IQR)	0.0240(0.03)	0.0415(0.03)	
Min - Max	0.0110 - 0.046	0.0160 - 0.059	

*Significant difference at *p* value≤0.05

Table 3: Comparison between the study and control groups regarding the thickness of hypertrophic layer

	Control Group	Study Group	Test (P value)
Mean (SD)	0.08 (0.02)	0.06 (0.004)	(0.027*)
Median (IQR)	0.09 (0.04)	0.06 (0.01)	
Min - Max	0.05 – 0.10	0.05 – 0.06	

*Statistically significant difference at *p* value≤0.05

Table 4: Comparison between the study and control groups regarding the concentration of serum vitamin A

	Control Group	Study Group	Test (P value)
Mean (SD)	235.50(4.04)	341.00(76.96)	0.03*
Median (IQR)	236.50(4.50)	345.00(53.00)	
Min - Max	230.00239.00 -	243.00431.00 -	

*Statistically significant difference at p value≤0.05

DISCUSSION

Vitamin A has been recognized to be essential for normal growth and bone health. However, many undesirable effects were associated with overdose of vitamin A. Unwanted effects of hypervitaminosis A on bone appears to be irreversible.(14, 25)

The effect of vitamin A on the condylar cartilage was not widely investigated. The present study was designed to explore the effect of over as well as optimum dose of vitamin A on growth of mandibular condyle.

In the methodology of the present study, four months old age rats were chosen as this age is correlated with 12years of human. This period is critical for the mandibular growth as it is characterized by mixed dentition in which replacement of deciduous teeth by their successor teeth occurs.

The result of the present study, the control group showed a normal structure and thickness of the fibrocartilage, proliferating, chondroblastic zones and hypertrophic cell zone. The osteogenic layer showed well-formed bone trabeculae with normal size of bone marrow.

The results obtained from the experimental group after 2 months revealed thickening of the chondroblastic zone and thinning of hypertrophic zone as compared to control group. These findings are in accordance with Onder et al,2018, who reported that hypervitaminosis A leads to reduction in the height of hypertrophic zone and increase in height of proliferative zone in rats exposed to high dose of vitamin A (26).

Thickening of chondroblastic zone and thinning of hypertrophic zone could be explained that hypervitaminosis A leads to retardation of transition between the chondroblastic zone and the hypertrophic zone. This retardation leads to delay of endochondral bone formation.

In addition to reduction of the hypertrophic cell zone, tearing of hypertrophic cell with signs of degeneration were observed. These findings are similar with studies that clarified that hypervitaminosis A induced chondroclast cells to absorb the calcified cartilage matrix and result in the erosions of hypertrophic zone(27, 28).Also, Firat.D, Kuntsal .L and Sirin. Y,2005, had reported that Retinoic acid induced chondrocytes degeneration of fetal Meckel's cartilage(29).

As regard to osteogenic zone thin bone trabeculae with wide bone marrow were observed as a sign of osteoporosis. Similar findings had been recorded by Lind T.et al, 2011, who reported that over dose of vitamin A increases osteoclasts number and leads to thin weakened long bone(30).In human models, it had been reported that excessive vitamin A has been implicated in the acceleration of skeletal bone resorption and inhibition of bone formation and ultimately leading to bone loss(31) .Areas of woven bone were observed in the study group and theses represent delay in maturation of the bone in rats with hypervitaminosis A.

The histomorphometric measurement revealed the total surface area of bone in the control group was higher than in the study group and it was statistically significant. These findings were in accordance with several studies that reported decrease in total surface area of bone in animal with hypervitaminosis A(13, 32).

As regard to histomorphometric measurement of chondroblastic zone. There was no significant difference between the control group and study group despite the mean value of thickness were higher in study in comparison to control. In contrast, measurements of hypertrophic layer revealed significant reduction in thickness in study group compared to control group. These findings were in agreement with Onder et al, 2018,who found that there was significant reduction in the hypertrophic zone without

significant increase in thickness of chondroblastic zone in the groups administered over dose of vitamin A(26).

As concern to quantitative determination of serum vitamin A concentrations, all rats showed elevated level of serum vitamin A concentration in the study groups as compared to rats in the control groups and it was statistically significant. These finding were in agreement with Lind et al,2018, who studied the effects of hypervitaminosis A on rat bone and recorded elevated level of serum vitamin A in study group as compared to control group and it was statistically significant (33).

In the present study, the control group showed normal structure of cartilage and bone in the light microscope examination while the study groups showed marked osteoporosis and retardation of endochondral bone growth.

This effect could be explained by effect of vitamin A on the actions of vitamin D. Over dose of vitamin A may inhibit vitamin D absorption, transport, or conversion to its active form, or it may promote vitamin D metabolic breakdown (34). Since the main function of vitamin D is enhance the absorption of calcium in the intestine, the over dose of vitamin A leads to decrease serum calcium level(34). Low serum calcium levels increase the production of parathyroid hormone (PTH), which helps to restore normal serum calcium levels by releasing calcium from the bone and promoting bone turnover (35).

An excessive dose of vitamin A was found to stimulate bone resorption by its stimulatory effect on the osteoclast cell. In these aspects the action of vitamin A resemble that of parathyroid hormone(36). These results were in accordance with lind,et al,2011,(30)in vitro study and Johansson, et al,2002, (37)in vivo study, who reported that an overdose of vitamin A stimulated bone resorption (37) .In some animals, this resorption may be so extensive that it causes spontaneous fractures (30).

CONCLUSION

Hypervitaminosis A leads to increase resorption of the bone and diminish endochondral bone growth by premature closure of the condylar cartilage.

Conflict of interest

We declare that we have no conflicts of interest.

Funding

The authors received no specific funding for this work.

REFRENCES

1. .D'Ambrosio DN, Clugston RD, Blaner WS. Vitamin A metabolism: an update. Nutrients. 2011;3(1):63-103.
2. Harrison EH. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2012;1821(1):70-7.
3. Fennema OR. Fennema's food chemistry: CRC press; 2008.
4. Polcz ME, Barbul A. The role of vitamin A in wound healing. Nutrition in Clinical Practice. 2019;34(5):695-700.
5. Henning P, Conaway HH, Lerner UH. Retinoid receptors in bone and their role in bone remodeling. Frontiers in endocrinology. 2015;6:31.
6. Organization WH. Xerophthalmia and night blindness for the assessment of clinical vitamin A deficiency in individuals and populations. World Health Organization; 2014.
7. Mahassni SH, Al-Shaikh NA. Effects of vitamin A overdose on the immune system in rats. International Journal of Pharma Medicine and Biological Sciences. 2013;2(4):80-91.
8. Blomhoff R. Vitamin A and carotenoid toxicity. Food and Nutrition Bulletin. 2001;22(3):320-34.
9. Dawson M. The importance of vitamin A in nutrition. Current pharmaceutical design. 2000;6(3):311-25.
10. Penniston KL, Tanumihardjo SA. The acute and chronic toxic effects of vitamin A. The American journal of clinical nutrition. 2006;83(2):191-201.
11. Sale TA, Stratman E. Carotenemia associated with green bean ingestion. Pediatric dermatology. 2004;21(6):657-9.
12. Takita Y, Ichimiya M, Hamamoto Y, Muto M. A case of carotenemia associated with ingestion of nutrient supplements. The Journal of dermatology. 2006;33(2):132-4.
13. Lind T, Öhman C, Calounova G, Rasmusson A, Andersson G, Pejler G, et al. Excessive dietary intake of vitamin A reduces skull bone thickness in mice. PLoS One. 2017;12(4):e0176217.
14. Binkley N, Krueger D. Hypervitaminosis A and bone. Nutrition reviews. 2000;58(5):138-44.
15. Michaëlsson K, Lithell H, Vessby B, Melhus H. Serum retinol levels and the risk of fracture. New England Journal of Medicine. 2003;348(4):287-94.
16. Avery JK, DJ C. Temporomandibular Joint. In: Essentials of oral histology and embryology: a clinical approach. 3rd ed ed. St. Louis, Mo.:Mosby Elsevier2006.
17. .Delatte M, Von den Hoff JW, Van Rheden RE, Kuijpers-Jagtman AM. Primary and secondary cartilages of the neonatal rat: the femoral head and the mandibular condyle. European journal of oral sciences. 2004;112(2):156-62.
18. .Nistiar F, Racz O, Lukacinova A, Hubkova B, Novakova J, Lovasova E, et al. Age dependency on some physiological and biochemical parameters of male Wistar rats in controlled environment. Journal of Environmental Science and Health, Part A. 2012;47(9):1224-33.

19. De Bias J, Santoma G, Carabaño R, Fraga MJ. Fiber and starch levels in fattening rabbit diets. Journal of Animal Science. 1986;63(6):1897-904.
20. Council NR. Guide for the care and use of laboratory animals. 2010.
21. Kumar G. Preparation of Specimens for Histologic Study. In: Orban'sOral Histology & Embryology. 13th ed ed. India: Elsevier Health Sciences; 2014.
22. Kure-Hattori I, Watari I, Takei M, Ishida Y, Yonemitsu I, Ono T. Effect of functional shift of the mandible on lubrication of the temporomandibular joint. Archives of oral biology. 2012;57(7):987-94.
23. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. Journal of pharmacology & pharmacotherapeutics. 2010;1(2):87.
24. Egan KP, Brennan TA, Pignolo RJ. Bone histomorphometry using free and commonly available software. Histopathology. 2012;61(6):1168-73.
25. Tanumihardjo SA. Vitamin A and bone health: the balancing act. Journal of Clinical Densitometry. 2013;16(4):414-9.
26. Onder S, Bilgili SG, Bulut G, Celik HT, Oguztuzun S, Onder H, et al. Biochemical and histopathologic assessment of effects of acitretin on epiphyseal growth plate in rats. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii. 2020;37(3):346.
27. SOETA S, MORI R, KODAKA T, NAITO Y, TANIGUCHI K. Histological disorders related to the focal disappearance of the epiphyseal growth plate in rats induced by high dose of vitamin A. Journal of Veterinary Medical Science. 2000;62(3):293-9.
28. KODAKA T, TAKAKI H, SOETA S, MORI R, NAITO Y. Local disappearance of epiphyseal growth plates in rats with hypervitaminosis A. Journal of veterinary medical science. 1998;60(7):815-21.
29. Firat D, Kuntsal L, Sirin Y. Protective effects of prenatal administration of folic acid on retinoic acid-induced cellular damages of Meckel's cartilage in rats. The Tohoku journal of experimental medicine. 2005;205(1):27-36.
30. Lind T, Lind PM, Jacobson A, Hu L, Sundqvist A, Risteli J, et al. High dietary intake of retinol leads to bone marrow hypoxia and diaphyseal endosteal mineralization in rats. Bone. 2011;48(3):496-506.
31. Kawahara TN, Krueger DC, Engelke JA, Harke JM, Binkley NC. Short-term vitamin A supplementation does not affect bone turnover in men. The Journal of nutrition. 2002;132(6):1169-72.
32. Lind T, Sundqvist A, Hu L, Pejler G, Andersson G, Jacobson A, et al. Vitamin a is a negative regulator of osteoblast mineralization. PLoS One. 2013;8(12):e82388.
33. Lind T, Lind PM, Hu L, Melhus H. Studies of indirect and direct effects of hypervitaminosis A on rat bone by comparing free access to food and pair-feeding. Upsala journal of medical sciences. 2018;123(2):82-5.
34. Rohde CM, Manatt M, Clagett-Dame M, DeLuca HF. Vitamin A antagonizes the action of vitamin D in rats. The Journal of nutrition. 1999;129(12):2246-50.
35. Chertow B, Williams G, Norris R, Baker G, Hargis G. Vitamin A stimulation of parathyroid hormone: interactions with calcium, hydrocortisone, and vitamin E in bovine parathyroid tissues and effects of vitamin A in man. European journal of clinical investigation. 1977;7(4):307-14.
36. Frankel TL, Seshadri MS, McDowall DB, Cornish CJ. Hypervitaminosis A and calcium-regulating hormones in the rat. The Journal of nutrition. 1986;116(4):578-87.
37. Johansson S, Lind P, HÅKansson H, Oxlund H, Melhus H. Subclinical hypervitaminosis A causes fragile bones in rats. Bone. 2002;31(6):685-9.