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THE HISTOPATHOLOGICAL CHANGES IN RAT FEMUR THAT WERE FED WITH LOW-CALCIUM DIET IN LACTATION AND POSTLACTATION PERIODS *

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The purpose of the present study was to investigate structural changes of the femur of lactating and postlactation periods fed low-calcium diet in rats.Twenty pregnancy Wistar albino rats weighing between 200-250 g were used for this study.The laboratory animals divided into two groups consist of 10 female rats.The experimental group was fed with low-calcium diet for 90 days (0.083 % Ca).The puppy rats in this group following lactation and postlactation periods were fed with same diet.The control group was fed with standard diet (Standard pellet-0.5%Ca).

At the end of the experimental feeding period, the animals were sacrified and their femurs, removed enbloc sections (5μm) were taken and stained (Hematoxylen-Eosin, Masson Trichrome, Safranin fast green) for the light microscope. Light microscope examination; in the puppy rats in low-calcium diet group some histological sections of bone showed demineriliz ation area, an irregularitiy of inner surface of the lamellae and increase of the number of osteoclasts. Reducing the thickness of bone cortex and spongiosae trabeculae result from resorbtion were noted.
Key words: *Low-calcium diet, femur, lactation, postlactation period, rat*

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laktasyon ve laktasyon sonrasi dönemde düşük kalsiyum diyetle beslenen sıçanların femurundaki histopatolojik değişiklikler

Çalışmamızda laktasyon ve laktasyon sonrası düşük kalsiyum diyetle beslenen ratların femur kemiğindeki histolojik değişiklikleri araştırdık. Araştırmamızda 20 tane erişkin 200-250 g ağırlığındaki Wistar albino rat kullanıldı.

Kontrol grubundaki ratlar çiftleştirmeye alındıkları günden itibaren standart pelet yem ile beslendi (%0,5 Ca içeren).Bu gruptan elde edilen ratlar, laktasyon ve laktasyon sonrası periyotta da aynı diyetle beslendi. Deney grubundaki dişi ratlar, çiftleştirmeye alınarak gebelik döneminde standart pelet yem ile beslendi (%0,5 Ca içeren). Deney grubunun yavru ratları laktasyon ve laktasyon sonrası düşük kalsiyum içeren (%0.083 Ca içeren) diyetle beslendi. Deney hayvanları 90.günde eter aneztezisi altında sakrifiye edilerek sağ femurları alınarak, %10'luk nötral formalinde fikse edildi. Femurların dekalsifikasyonu % 5'lik formik asitle yapılarak, rutin histolojik takiplerle elde edilen 5μ kalınlığındaki parafin kesitleri Hematoxylen-Eosin, Masson trichrome, Safranin fast green ile boyandı.

Yavru ratların femur kesitlerinde; kompakt kemikte demineriliz asyon alanları ve lamellerde düzensizlikler ile osteoklast sayısında artış gözlendi.Rezorpsiyon sonucu, kemik korteksi ve spongiöz trabeküllerde belirgin bir incelme saptandı.
Anahtar Kelimeler: *Düşük kalsiyum, femur, laktasyon, laktasyon sonrası, rat*

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During lactation a 10-20 % loss of the total ash content from calcified bone has been reported. This which is due to exclusively bone eresion, but not to demineralization. In response to calcium insufficiency in the diet, the skeleton of these animals may release up to 50% of Ýts components, which especially at with the ends of long bone most severely affected[1,2,3] .

The fact that osteoprosis due to an extreme demineriliza tion from both a Ca-deficient diet at lactation, is less pronounced in compact then spongy regions of the skeleton indicates that osteoclast activity, is coverned not only by variations in serum calcium-active hormone levels but also by local factors.The nature local factors is not known.However,since spongy has more extensive free surface than compacta, many workers suggested that the greater tendency of the spongiosa to lose bone tissue is related to architecture of the tissue[4] .

On the other hand, the calcium ion is a major substance of intracellular messenger system in cell division and cell differention and is used in bone tissue for mineralization[5] . Hypovitaminosis D and a low calcium deficiency in growing rats causes osteoporosis and inhibits skeletal growth [6] . Serum Ca levels vere decreased in the calcium deficient diets[7] . Low-Ca diet reduced bone mineral density, i.e., induced osteoporosis [8] .

Some investigatators have evaluated the amount and distribution of bone tissue resorption in several compact and spongy region of the femora from lactating rats maintained on a normal or low-calcium diet to determine whether the differences in osteopenia between compact and spongy regions are exclusively the result of the greater extent the free surface in the latter[9] .

This study aims to investigate the histopathological changes in femur from the lactation and postlactation periods fed a low-calcium diet in rats.

MATERIALS AND METHODS

60 days of age when the experiment began twenty 200-250 g female rats (Wistar albino strain, Health Medical Research Center-DÜSAM, , Diyarbakır -TURKEY), were divided in the two groups of 10 as follows:

Control group; given a well-balanced optimal diet (Control diet). These lactating females were fed diet with normal calcium content (0.5% Ca-Table-I).The control group was fed with standart diet for 90 days (0.5% Ca).

Experimental group; The pregnancy rats were fed with standard diet for 22 days (0.5% Ca).The experimental group was fed with low-calcium diet for 90 days (0.083 % Ca-Table-II). The puppy rats in this group following lactation and postlactation periods were fed low-calcium diet (10). Each group was given their respectively diet and deionized water add libitum for 90 days.

Table-I: Standard Diet

Nutrient	**Concentration in a diet (%)**
Dry Substance	89.23
Water	10.77
Crude Protein	16.114
Crude Cellulose	3.24
Crude Ash	4.73
Fat	4.77
Calcium (Ca)	0.5 *
Phosphorus (P)	0.4
Sodium (Na)	0.08
Salt (NaCl)	0.1
Methionin+Cysteine+Lysine	0.7
Digestibel Energy (D.E.)	3065.45 kcal/kg

Tablo-II:Low-Calcium Diet

Nutrient	**Concentration in a diet (%)**
Dry Substance	89.23
Water	10.868
Crude Protein	16.24
Crude Cellulose	3.27
Crude Ash	4.73
Fat	4.79
Calcium (Ca)	0.083*
Phosphorus (P)	0.406
Sodium (Na)	0.08
Salt (NaCl)	0.1
Methionin+Cysteine+Lysine	0.906
Digestibel Energy (D.E.)	3098 kcal/kg

All rats were sacrified by decapitation under ether anesthesia at the end of experimental periods.The right femurs were dissected immediately,adherent tissues were well removed,and bones were then fixed in 10% neutral formalin for two days at 4°C.

For histopathologic examinations, the right femur were decalcified in 5% formic acide, embeded in paraffin by the routine procedure,and sectioned longitudinally at tickness 5 μm[11] .The lonigitudinal sections were stained with hematoxylin and eosin,Trichrome Masson, Safranin Fast Green. The specimens were evaluated with the light microscope.

RESULTS

The section of the femur in control group fed with the standard pellet was not determined any pathological findings (Figure-1).

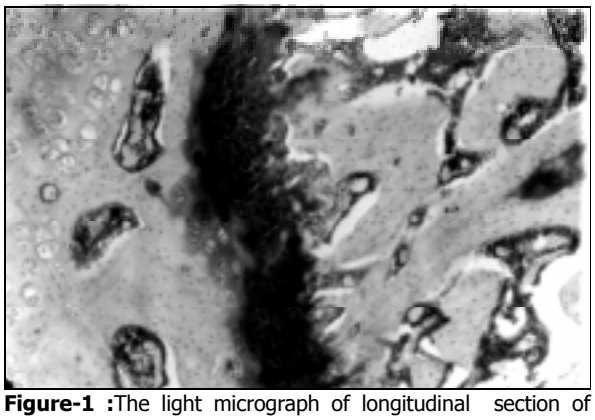

Figure-1 : The light micrograph of longitudinal section of femur of control rat showing proximal epiphyseal plaque (e) and trabeculae (t) (Safranin-Fast Green,original magnification X41).

The puppy rats in low-calcium diet group some histological sections of the bone showed demineralization area, an irregularity of inner surface of the lamellae and an increase of the number of osteoclasts (Figure-2a).

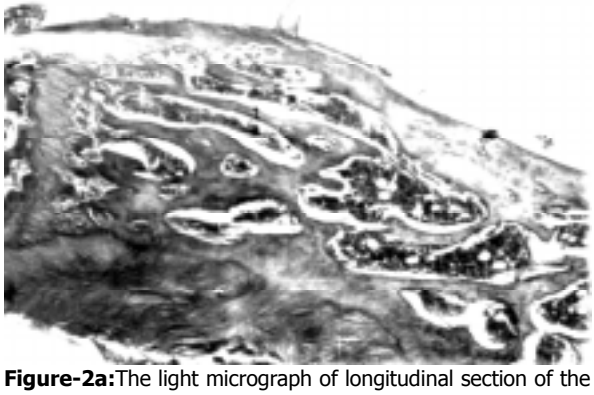

Figure-2a: The light micrograph of longitudinal section of the femur of a new born rats.Note an increase of the thickness of epiphyseal cartilage(e) and bone trabeculae (t) (Masson trichrome, original magnification X41).

Both irregularities of compact bone lamellae and demineralization area were seen. An increase of both number of osteoblast and osteoclasts were prominent. Reducing the thickness of bone cortex and spongiosa trabeculae result from resorbtion were noted (Figure-2b).

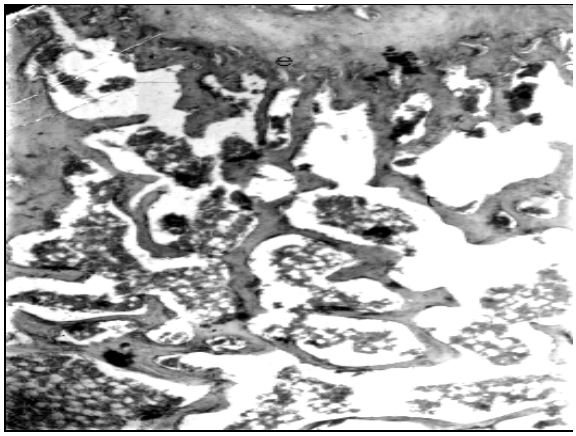

Figure-2b : The light microscopic view: longitudinal section of femur of a newborn rat.Note reducing the tickness of spongiosa trabecullae (t) (Hematoxylen-Eosin,original magnification X16).

DISCUSSION

Animal studies have showed that the number of trabeculae in spongiosa is reduced in rats fed low-calcium diet. Different disorders and inadequate calsification are observed in the skeleton and osteocytes are bigger than normal and unstained in rats fed diet without calcium [3,9] , are paralel with our findings.

Rats fed on a low-Ca diet exhibit hypocalcemia and the decreased with of the proliferative zone may be due to inhibition of chondroblastes and/or their progenitor cells[5] .

The decreased intracellular glycogen storage together with less developed cartilage cell might indicate an inhibition of chondroblast cytodifferentiation and maturation in the low-Ca rats, is in harmony with that of[5] results.

The histologic findings showed that ossification of the mandibular ramus was clearly inhibited in the low-Ca group. What we found about this conformes with the findings of Nagayama et al [12] .

Because of inadequate mineralization the adulthood, bone trabeculae became thin. The

cortex became thin as a result of subperiosteal and endosteal resorbtion and also haversian systems became wide[9,12] . Same results were found rat femur's sections in our study (Figure-2a,b).

Hipocalcemia causes to increase the osteoclastic activity and inadequate of mineralization of bones in the adolescent and adulthood. It is also causes backwardness as result of inadequate calsification in epiphyseal cartilage and disorders in the endochondral bone growing[13] is paralel with our results.

These results also suggest that : (a) Decrease the thickness of spongiosa. (b) Demineralization of trabecullae and compact bone. (c) Increase the number of osteoclasts in the trabeculae and compact bone of low-calcium group.

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