

PP08: Intrauterine growth restriction affects bone mineral density of the mandible and the condyle in growing rats

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Introduction

Intrauterine Growth Restriction (IUGR) has been associated with increased prenatal and postnatal morbidity and mortality, while also linked with a variety of maternal-fetal pathological conditions. Furthermore, infants may fail to reach their expected growth potential. The present study investigates the effect of IUGR on the quality of the mandibular and condylar bone and density of the mandible and tibia of growing rats.

Materials and Methods

Six first-time pregnant Wistar rats were obtained at 12 days of gestation; 3 of the mothers underwent a model of 50% food restriction until the end of pregnancy (21 days) and 3 of the mothers were fed *ad libitum*. The pups were culled to 8 (4 males and 4 females) per litter to normalize rearing. The offspring groups of investigation were: Group A: 12 male rats born IUGR and Group B: 12 Control male rats. Both groups were postnatally fed *ad libitum*. The experiment ended when the male rats reached 150 days of age. Dual-energy X-ray absorptiometry (DEXA) was performed for the whole tibia and proximal tibia metaphysis when the rats were 60 and 150 days old. DEXA of the mandible, examination of biochemical markers, and histology were performed after sacrifice. Finally histomorphometrical analysis was done on the mandibular bone Region of Interest (mROI), the subchondral bone of the condyle (cROI) and for the thickness of the condylar cartilage layers (IROI).

Results

DEXA for tibia and proximal tibial metaphysis. Two-way ANOVA revealed statistically significant differences between groups for both total and proximal tibia at 2 and 5 months. Bone mineral density (BMD) at 2 months for tibia bone, for IUGR was 0.171 ± 0.008 vs 0.186 ± 0.005 for Control ($p < 0.005$). At 5 months the respective values were 0.252 ± 0.015 for IUGR vs 0.274 ± 0.007 for Controls ($p < 0.005$). For the proximal tibial metaphysis, at 2 months BMD was 0.237 ± 0.022 for IUGRs vs 0.266 ± 0.029 for Control ($p = 0.017$) while at 5 months the respective values were 0.360 ± 0.018 for IUGRs vs 0.404 ± 0.021 for Controls ($p < 0.005$). All values are presented as mean \pm SD mg/cm².

DEXA in the mandible in two specific regions: Area 1 at 2 mm above the antegonial notch and Area 2 at 2mm above Me (menton). After using independent t-Test IUGR and Control groups, were compared both at 5 months of age, BMD was estimated in Area 1 for IUGR 0.233 ± 0.031 vs 0.290 ± 0.021 for Controls ($p < 0.0005$). In Area 2 BMD was estimated for IUGR 0.308 ± 0.029 vs 0.350 ± 0.029 for Control ($p = 0.003$) (Figure 1,2). Statistical t-Test analysis was performed for **Osteocalcin** in ng/ml for IUGR 162.12 ± 50.05 vs 207.94 ± 30.38 for Controls ($p = 0.021$). For **Vit D** (μ g/ml) for IUGR 1028.62 ± 134.20 vs 1098.07 ± 180.53 for Controls ($p = 0.352$).

Phosphorus (mg/dL) for IUGR is 22.40 ± 3.27 vs 27.77 ± 6.01 for Controls ($p = 0.028$).

Histomorphometrical analysis of the specimens revealed that percentage of bone in mROI for IUGRs was 51.21 ± 5.54 vs 66.00 ± 15.49 for Controls $p = 0.007$. The percentage of marrow spaces in mROI for IUGRs was 48.79 ± 5.54 vs 34.01 ± 15.49 for Controls. (Figure 3,4,7). The percentage of bone cROI for IUGRs was 47.01 ± 6.82 vs 68.27 ± 13.37 for Controls $p < 0.005$. The percentage marrow spaces in cROI for IUGRs was 53.00 ± 6.82 vs 31.73 ± 13.37 for Controls (Figure 5,6,8). IROI for the IUGR presented statistically significantly lower values of **Fibrous layer** (20.48 ± 3.45 vs 26.21 ± 7.79 , $p = 0.036$), higher values of **Hypertrophic layer** (50.12 ± 6.31 vs 43.74 ± 7.93 , $p = 0.05$) and no difference for **Proliferative layer** (29.40 ± 5.49 vs 30.05 ± 6.39 , $p = 0.802$) compared to Control group (Figure 9,10).



Figure 1: Area 1 above the antegonial notch and area 2 above Menton, the right hemi-mandible.

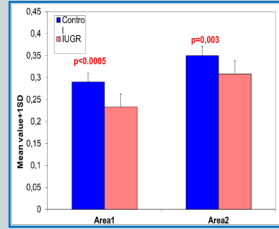


Figure 2: BMD of the mandible of both groups in different Areas. The bars represent standard deviation

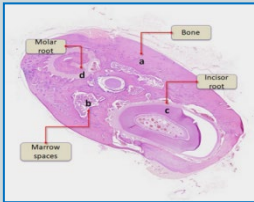


Figure 3: Transverse section of mandibular bone a) bone, b) marrow spaces, c) incisor root, d) molar root.

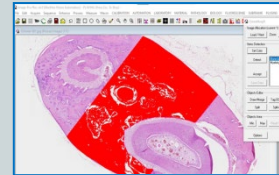


Figure 4: Bone in the mROI is colored red (hematoxylin-eosin staining, initial magnification x20)

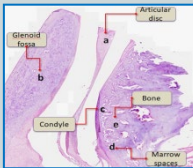


Figure 5: Section in the sagittal plane of the condyle. a) Articular disc, b) glenoid fossa, c) condyle, d) marrow spaces, e) bone.

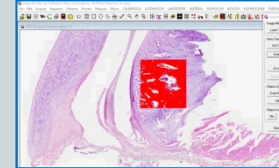


Figure 6: Histomorphometrical analysis of the subchondral bone of the condyle, bone in the cROI is colored red

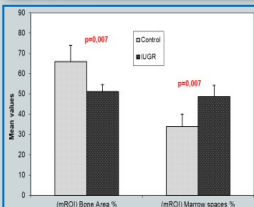


Figure 7: Comparison of Histomorphometrical results of the mandibular bone (mROI) between IUGR and Control group for Bone area and Marrow spaces

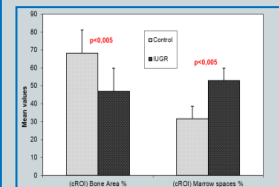


Figure 8: Comparison of Histomorphometrical results of the condylar bone (cROI) between IUGR and Control group for Bone area and Marrow spaces.

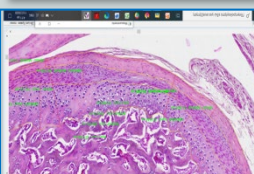


Figure 9: Histomorphometrical analysis of the thickness of the condylar cartilage in (IROI) and percentage determination of each layer separately (fibrous, proliferating and mature chondrocyte layer -hypertrophic cartilage layer)

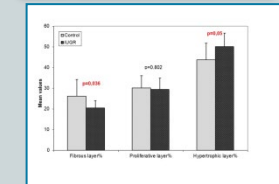


Figure 10: Comparison of Histomorphometrical results for thickness of the condylar cartilage layers between IUGR and Control group

Conclusion

Our results provide original information regarding the quality and density of the mandibular bone in growing rats and indicate that maternal restricted nutrition during gestation can affect the bone density of the mandible. Moreover there was no recovery of BMD at the end of the experiment after normalization of the diet.