Mandible Growth of Wistar Rats: Effects of Malnutrition during Lactation in Adulthood Mandibular Size

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Abstract

Introduction Malnutrition is a world health issue; thus, it is imperative to know its effects during lactation. The aim of this study was to evaluate the effects of maternal protein restriction during lactation on the horizontal and vertical bone growth of the mandible, according to predetermined parameters.

Material and Methods At parturition, Wistar rat dams were randomly assigned to the following groups: (1) control, which had free access to a standard laboratory diet containing 23% of protein; (2) protein energy-restricted, which had free access to an isoenergetic, protein-restricted diet containing 8% of protein; and (3) energy-restricted group, which received limited access to commercial diet containing 23% of protein. After 21 days, all pups received free access to a standard laboratory diet containing 23% of protein until their adulthood. Afterwards, the animals were euthanized under thiopental anesthesia and their mandibles were excised. The dimensions of the excised pup mandibles were measured directly with a digital caliper.

Results The protein-restricted and the energy-restricted groups presented minor adulthood mandibular length in all parameters analyzed.

Conclusion Our results provide original information regarding the mandibular growth and indicate that the maternal nutritional state during lactation can affect the development of mandibular growth. Moreover, our results indicated that the growth dysfunction could not be restored after normalization of the diet.

Keywords
- lactation
- malnutrition
- mandible
- growth and development
- wistar rats

Introduction

Some investigators have shown that the nutritional status of the mother during gestation and lactation is essential for normal growth and development, both in human and in experimental animals.[1,2]

Previous studies have shown that maternal undernutrition during lactation can cause alterations in breast milk composition,[2] and serum hormone concentrations,[3] which can lead to a reduction in body weight.[2,3]

Rats are used as a model for studying human skeletal system and major bone diseases and are considered as a good model for nutritional research.[2,4–10]

Nonetheless, few studies have focused on mandibular growth, especially regarding its relation to nutritional status [4,8]

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Developmental mandibular abnormalities, both in the horizontal and in the vertical directions, are the most common components of malocclusion.[11]

We consider that this is an important aspect in the field of mandibular development research, as procedures involving this region are emerging and becoming more complex. Also, the study of protein malnutrition effects on mandible is worthwhile, considering the prevalence of this condition in patients, especially in developing countries.[1,12,13]

Thus, the objective of this study was to evaluate the effects of maternal protein restriction during lactation on the horizontal and vertical bone growth of the mandible of the pups in adulthood.

Material and Methods

Animal Care

The handling of the animals was approved (CEUA/ 036/2010) by the Animal Care and Use Committee of Universidade do Estado do Rio de Janeiro, which based their analysis on the Guide for the Care and Use of Laboratory Animals.[14] This study was conducted from April 1, 2010 to June 1, 2012.

Experimental Model

Six virgin female Wistar rats (3-month-old) were kept in a room with controlled temperature (25 ± 1°C) and with artificial dark-light cycle (lights on from 07:00 hours to 19:00 hours) all throughout the experiment and were caged with three male rats. After mating, each female rat was placed in an individual cage with free access to water and food until delivery.

The pregnant Wistar rats were randomly separated at delivery into three groups (two dams per group): group 1 control (C) had free access to water and a standard laboratory diet (in grams per 100 g) containing 23% of protein, 68% of carbohydrate, 5% of lipids, 4% of salts and 0.4% of vitamins, 4070.4 total energy (kJ/kg); group 2 - protein energy restricted (PER) had free access to water and to an isoenergetic, protein-restricted diet containing 8% of protein; and group 3 - energy-restricted (ER) received free access to water and limited access to commercial diet containing 23% of protein, which corresponded to the same amount ingested in the previous day by rats in group PER. The number of pups used was 6 for each female rat, which totaled 12 per group.

The PER group consumed 60% of the amount consumed by the control group, in spite of having free access to water and laboratory food. Therefore, the amount of food consumed in both ER and PER groups was almost the same. The low-protein diet was prepared in our laboratory, and vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets.[16] The compositions of both diets are depicted in ►**Table 1**.

To evaluate the nutritional state, the food consumption and body weight were monitored throughout the experiment. Within 24 hours of birth, excess pups were removed, so that only six female pups were kept per dam, as it has been shown that this procedure maximizes lactation performance.[14]

Table 1 Diet composition

Ingredients (g/kg)	Control‡	PER§
Total protein†	23.0	8.0
Corn starch	676.0	826.0
Soybean oil	50.0	50.0
Vitamin mixture	4.0	4.0
Mineral mixture	40.0	40.0
Macronutrient composition (%)	**Control‡**	**PER§**
Protein	23.0	8.0
Carbohydrate	66.0	81.0
Fat	11.0	11.0
Total energy (kcal)	4070.4	4070.4

Abbreviation: PER, protein energy restricted.
†Principal protein resources were soybean wheat, steak, fish and amino acids.
‡Standard diet for rats (Nuvilab-Nuvital, Curitiba, Paraná, Brazil).
§The PER diet was prepared in the laboratory at Universidade Federal do Rio de Janeiro by replacing part of the protein content of the control diet with cornstarch. The amount of the latter was calculated to replace the same energy content of the control diet. Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves et al., 1993).[16]

The maternal malnutrition during lactation was started at birth, which was defined as day 0 of lactation and was ended at weaning (21-days-old). After weaning, 12 female pups of the same treatment group were housed in groups of 3 animals per cage (12 pups per group), and given unlimited access to food and water until adulthood (90-days-old); then, the animals were euthanized under thiopental anesthesia (0.15 mL per 100 g of body weight) and the left ventricle was perfused with buffered saline followed by formalin solution.

The mandibles were excised after perfusion, and they were dissected and stored in formalin solution (10%).

Morphometric Parameters

The horizontal and vertical bone growth of 36 mandibles (12 per group) was measured as defined in ►**Table 2**. The parameters are also illustrated in ►**Figs. 1** and **2**. All measurements in millimeters (mm) were made to the nearest 0.01 mm using a Mitutoyo digital caliper (Mitutoyo Corp., Kawasaki, Kanagawa, Japan). Both sides of the mandibles were measured.

Statistical Analysis

The data are reported as mean ± standard deviation (SD). The statistical significance of the experimental observations was determined using one-way analysis of variance (ANOVA) followed by the posttest of Newman-Keuls to compare the three experimental groups. The level of significance was set at $p \leq 0.05$. A comparison between sides was performed with the Student *t*-test ($p < 0.05$) and was considered statistically significant. The statistical analysis was performed using the GraphPad Prism 5 statistical software (GraphPad, San Diego, CA, USA).

Table 2 Parameters used in the morphometric analysis

Parameter	Definition
Length measures	
Length L1	Incisal edge of the lower central incisor tooth - most posterior point of the posterior border of the mandibular angle
Length L2	Lingual border of the alveolar process of the lower central incisor tooth - most posterior point of the posterior border of the mandibular angle
Length L3	Incisal edge of the lower central incisor - anterosuperior junction of mandibular body with the mandibular ramus
Length L4	Lingual border of the alveolar process of the lower central incisor - anterosuperior junction of mandibular body with the mandibular ramus
Height measures	
Height H1	Height of the alveolar process of the lower central incisor measured immediately ahead of the mesial of the alveolar process of the first inferior molar tooth (perpendicular to the occlusal plane)
Height H2	Lowest point of the sigmoid notch - Notch antagonist
Height H3	Anterosuperior junction of the mandibular body with the mandibular ramus - Notch antagonist

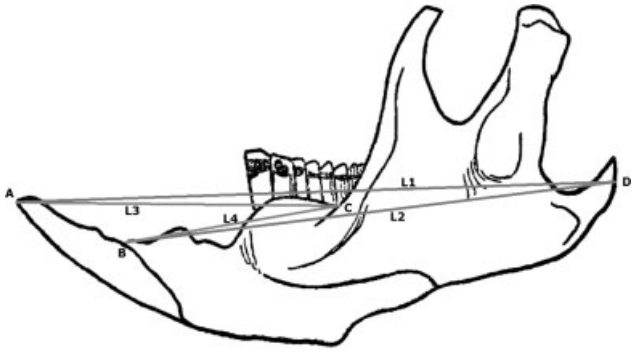

Fig. 1 Rat mandible showing the measurements (length) used in the morphometric analysis. Definitions of acronyms (L1 = length 1, L2 = length 2, L3 = length 3, L4 = length 4) are given in ►**Table 2**. Lateral view.

Results

The mandibular width and height results are shown in ►**Table 3**. These values showed significant differences when analyzing the mandibular body in both experimental groups compared with control. There were no differences between the left and right measurements of the mandibles ($p > 0.05$).

The evaluation of the total length of the mandible showed no significant differences, even on the ramus height, thus suggesting a developmental impairment of the mandibular body.

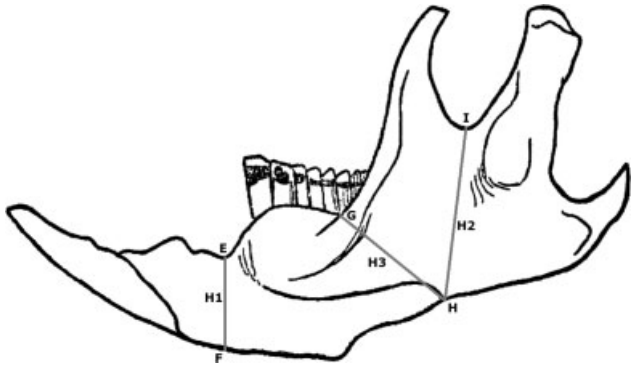

Fig. 2 Rat mandible showing the measurements (height) used in the morphometric analysis. Definitions of acronyms (H1 = height 1, H2 = height 2, H3 = height 3) are given in ►**Table 2**. Lateral view.

Discussion

The strengths of this study may be appreciated in its experimental design, the longitudinal nature of data collection, easy comparison to existing studies [5,10] using identical animal husbandry protocols,[4,10] and simultaneous contrast of targeted and catch-up growth.

The craniofacial skeleton is one portion of the body that is critically affected by malnutrition.[5,6,9,17] The skull is not a single developing unit, but rather has two distinct regions, the viscerocranium and the neurocranium.[6,18] The viscerocranium is used during the feeding and breathing mechanisms, and its growth is continuously subject to muscular loading, whereas the neurocranium houses the brain, and its growth is influenced primarily by brain expansion.[18–20]

Several studies have examined the effect of nutritional deficiencies on bone growth during gestation,[9] lactation,[6] gestation and lactation,[19] and the post weaning period.[21] Different forms of retarded cranial growth have been reported, depending on the type of malnutrition and/or its intensity, as well as the period in which the stress was applied. Additionally, the growth of the craniofacial components in rats may be influenced by sex, breed or strain, and nutritional status.[9,21] In the present study, we used same parameters of our previous study.[5]

Mandible underdevelopment was evident in weaned rats whose mothers were fed PER or ER diets during lactation. In our study, we observed that there was a failed catch-up growth by realimentation until adulthood (90-day-old). We also observed that abnormalities during the lactation period (21-day-old) remained until adulthood.

Table 3 Morphometric analysis of mandible growth in rat pups at 90 Days

Parameters	Control group	ER group	PER group	*P*-value
Length 1	30.78 ± 0.25	28.35 ± 3.22	28.05 ± 2.15	0.09
Length 2	24.87 ± 0.47	23.21 ± 2.15	22.91 ± 1.60	0.17
Length 3	15.69 ± 0.55	14.41 ± 1.50	14.25 ± 0.88	0.04
Length 4	11.02 ± 0.32	10.09 ± 0.94	9.70 ± 0.74	0.02
Height 1	4.34 ± 0.12	3.49 ± 0.37	3.50 ± 0.38	0.005
Height 2	9.54 ± 0.46	9.05 ± 1.18	8.77 ± 0.60	0.17
Height 3	6.30 ± 0.49	5.53 ± 0.11	5.37 ± 0.17	0.002

Abbreviations: ER, energy restricted; PER, protein energy restricted.
Results are shown as mean ± standard deviation.

The literature on catch-up growth presents a confusing mosaic of results. Several published reports document failed catch-up growth,[21,22] even in malnourished rats (HERRING, 1993).[17] There is an equally massive literature documenting successful catch-up growth,[23,24] including cases of growth restriction that occurred prenatally [23] [24]. Because growth dynamics are multifaceted, methodological differences among studies prevent straightforward comparisons among these results. Recent reviews outline many of the variables affecting catch-up growth, including length of growth restriction, type of growth restriction, and ontogenetic timing.[25,26]

Abnormalities in mandibular development, both vertically and horizontally, are the most common components of malocclusion. Dental skeleton changes, when ranging in intensity from moderate to severe, cause functional (impaired speech, breathing and chewing) and aesthetics problems to patients.[27] These skeletal malocclusions are difficult to treat, and commonly require correction by means of combined orthosurgical treatments.[4]

To date, according to the literature, no studies regarding morphometric aspects were found; thus, no comparisons could be made with our study, in which we observed that malnutrition during the period of lactation substantially affected the length and height development of the mandibular body, even using a normal and balanced diet after weaning until the end of the 90-days experiment.

Comparing patients with normal occlusion, with class II dental malocclusion, mandibular length was a minor factor.[28] Patients with class II malocclusion exhibit abnormalities in mandibular development, both vertically and horizontally, as the skull grows mainly due to the growth of the skull base, the condyle and mandible's body.[27,29]

In this study, the total length of the mandible and the height of its ramus have been significantly affected. We highlight that the role of intercuspal occlusal control of craniofacial morphogenesis[25] and stomatognathic functions are influenced by the position of the mandible. Currently, one cannot see occlusion as dependent only on the dental contacts. The main consideration should be the interference pattern of bone growth components of the facial skeleton and skull base.[30] Studies find that 60% of patients with atypical swallowing also present body-axis disharmonies [28] and explain that the center of gravity of the head is anterior to the atlanto-occipital articulation, tending to move forward and downward, which would alter the shaft of the body, and its balance is directly affected by the position of the mandible.[30]

According to the statements above, mothers should be more carefully treated, especially in the current culture of fitness and fast weight loss after birth, which are both subliminally published in newspapers and television. Malnutrition during lactation compromised the development of the mandible's body in its normal length and height, even though a normal and balanced diet was administered after weaning. Since malnutrition is a prevalent condition, we may see a generation with postural problems, craniofacial growth problems [6] and many other derivatives.

Conclusion

Our results provide original information regarding the mandibular growth and indicate that the maternal nutritional state during lactation can affect the development of the mandible skeleton and that this could not be restored by an improvement of the diet; thus, the catch-up growth was not successful.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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