ORTHODONTIC IMPLICATIONS OF PROTEIN UNDERNUTRITION IN MANDIBULAR GROWTH. A CEPHALOMETRIC STUDY IN GROWING RATS

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ABSTRACT

The present experimental work analyzes the development of different mandibular units and its likely impact on the direction of mandibular displacement during facial development, and the relation between the mesiodistal dimension of the first, second, and third molars and the length of the mandibular corpus in a model of protein undernutrition with muscular atrophy in growing rats. Sixteen Wistar rats weaned at the age of 21 days were assigned to one of the following groups: control (fed a regular hard diet ad libitum) and experimental (fed a diet lacking in protein, corn flour, ad libitum). All the animals were euthanized five weeks after the onset of the experiment. Following resection of the mandibles, the mandibles were hemisected at the symphysis and fixed in 10% formalin. Remaining soft tissue was removed. Metallic landmarks were placed in the mental and mandibular foramens of one hemimandible of each rat. The hemimandibles were radiographed. The cephalometric study was performed on paper tracings of the projected image of the radiographs.

Both groups exhibited a slight increase in body weight (b.w) throughout the first ten days of the experiment. After this point, the undernourished group showed no further increase in b.w., and exhibited significantly lower b.w. than controls at the end of the experiment.

The cephalometric study showed that the length of the mandible as a whole, and of the condylar and angular processes was significantly lower in the undernourished group. In addition, significant differences in the vertical relation between the angular process to the mandibular corpus, the convexity of the angular process, and the ratio between total molar width (from the first to the third molar) and the length of the mandibular corpus were observed.

Key words: *Protein restriction, Mandible growth, Facial development, Orthodontics*

IMPLICANCIAS ORTODÓNCICAS DE LA DESNUTRICIÓN PROTEICA EN EL CRECIMIENTO MANDIBULAR. ESTUDIO CEFALOMÉTRICO EN RATAS EN CRECIMIENTO.

RESUMEN

Se presenta un trabajo experimental en el que se analiza el desarrollo de diferentes unidades mandibulares y su probable impacto en: la dirección del desplazamiento mandibular durante el desarrollo facial y en la relación entre las dimensiones mesiodistales de primero a tercer molar con la longitud del cuerpo mandibular en un modelo de desnutrición proteica y atrofia muscular de ratas en crecimiento.

Se utilizaron ratas Wistar destetadas a los 21 días de edad y separadas en dos grupos denominados control (alimentado con una dieta dura convencional ad libitum) y experimental (alimentado con una dieta deficiente en proteínas, harina de maíz, administrada ad libitum). A las cinco semanas de experiencia todos los animales fueron sacrificados, se disecaron las mandíbulas y se separaron a nivel de la línea media, se fijaron en formol al 10% y se removieron los tejidos blandos. A una hemimandíbula de cada animal se le realizaron marcas metálicas a nivel del agujero mentoniano y del agujero mandibular, posteriormente fueron radiografiadas. A partir de proyecciones de las radiografías se obtuvieron trazados sobre los que se realizó el estudio cefalométrico.

El peso corporal aumentó levemente en los primeros diez días de experiencia en ambos grupos. A partir de ese punto en el grupo desnutrido no se incrementó y fue significativamente menor que el grupo control al final del período experimental.

El estudio cefalométrico mostró que las longitudes mandibular, del proceso condilar y del proceso angular fueron significativamente menores en el grupo desnutrido. Se encontraron además diferencias significativas en los valores que definen la relación vertical del proceso angular al cuerpo de la mandíbula, en la convexidad del proceso angular y en la relación entre la longitud mesiodistal total de primero a tercer molar con la longitud del cuerpo mandibular.

Palabras clave: *Restricción proteica, Crecimiento mandibular, Desarrollo Facial, Ortodoncia.*

INTRODUCTION

The development of the maxillae is a highly complex process; the maxillae are therefore vulnerable to endogenous (genetic) and exogenous (environmental) factors. Although the shape and size of the head, face and dental arches are determined by genetic factors (1), craneo-facial development is affected by external factors during growth. Studies in the literature have reported certain habits, trauma, and premature extraction of deciduous teeth to alter normal growth direction and to reduce arch length, causing crowding (2). In addition, there are other factors, such as nutritional deficiencies, that may appear during growth and affect bone development, causing marked variations in bone shape and size. The mandible is no exception. Protein undernutrition induced by feeding weanling rats a diet lacking protein results in bone growth arrest and alterations in the size and biomechanical properties of the mandible (3, 4, 5, 6). However, the implications of these undernutrition-related alterations in the direction of mandibular displacement during facial development, and in the ratio between mesiodistal tooth size to dental arch length have not been evaluated to date.

Protein undernutrition in third world countries is associated to weaning in an extremely poor environment. It occurs rapidly as a result of protein deficient diets based almost solely on carbohydrates (7). Protein undernutrition rapidly affects muscle tissue. When the nutritional deficiency is prolonged, the catabolism of fats and muscle proteins increases considerably, resulting in progressive muscle atrophy (8, 9, 10). Within this context, it is evident that the mandible develops under the negative influence of the nutritional deficiency and muscle atrophy. It can therefore be inferred that malnutrition may affect facial growth.

The aim of the present study was to analyze the potential changes in the direction of mandibular displacement during facial development, and the ratio of mesiodistal molar size to mandibular arch length in a model of protein undernutrition in growing rats with different masticatory muscle capacities, by means of cephalometric evaluation.

MATERIALS AND METHODS

Sixteen newly born Wistar rats were used. The animals were weaned at the age of 21 days and assigned to one of the following groups: control group (n=8) fed a regular hard diet ad libitum (Table 1), and experimental group (n=8) fed a diet lacking protein (corn meal) ad libitum (Table 1). Body weight of all the animals was recorded throughout. Five weeks after the onset of the experiment (rats aged 8 weeks) the animals were euthanized, the mandibles were carefully dissected and fixed in 10% formalin, after which the soft tissue was removed.

In order to perform the radiographic study, the mandibles were hemisected at the symphysis. Metallic landmarks consisting of L shaped 0.2mm steel ligature wires (11) were placed in the mental and the mandibular foramens of one hemimandible of each rat (Figure 1). The marked hemimandibles were positioned laterally on the film next to a 10mm long wire and radiographed using standard X-ray equipment at 70Kv and 8mA, 0.3 sec exposure time; the focus to film distance was 40cm. Reference points were marked on paper tracings of the

TABLE 1. Chemical centesimal composition for each component (%) of the diets employed to feed the control and undernourished groups ME/Kg: metabolizable energy

	Control	Undernourished
Maximum Humidity	12%	10.9%
Proteins	23%	6.9%
Fiber	6%	13.4%
Total mineral content	10%	0.6%
Calcium	1.4%	0.007%
Phosphorous	0.8%	0.003%
Sodium	0.2%	0.005%
Potassium	0.7%	0.003%
Magnesium	0.2%	0.09%
Energy concentration	3100Kcal. ME/Kg	3610Kcal. ME/Kg

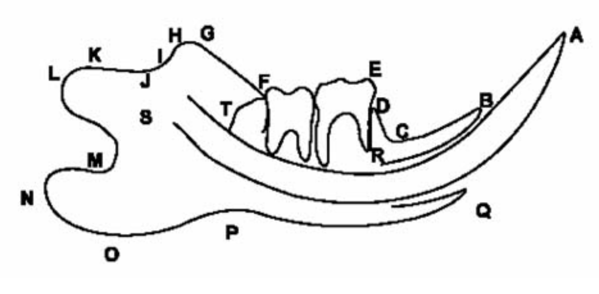

Fig. 1. Reference points defined on the lateral roentgenograph of the mandible, according to Kiliaridis S (11). A: The most prominent point of the incisal edge of lower incisors. B: Lingual alveolar crest of lower incisors. C: Intersection between the lower incisor alveolar process and lower first molar alveolar process. D: Alveolar process mesial crest of the lower first molar. E: Mesial buccal cusp of the lower first molar. F: Intersection between the occlusal plane and the anterior border of the mandibular ramus. G: The most anterior and superior point of the coronoid process. H: The most posterior and superior point of the coronoid process. I: The most anterior point of the mandibular notch. J: The most inferior point of the mandibular notch. K: Fovea pterigoidea. L: The most posterior point of the condylar process. M: The deepest point of the posterior notch in the ramus of the mandibles. N: The most posterior point of the angular process of the mandible. O: The most inferior point of the lower border of the angular process. P: The deepest point of the inferior border of the mandible. Q: Vestibular alveolar crest of lower incisors. R: Position of markers in mental foramen. S: Position of markers in mandibular foramen. T: The most posterior point of the distal face of the third molar.

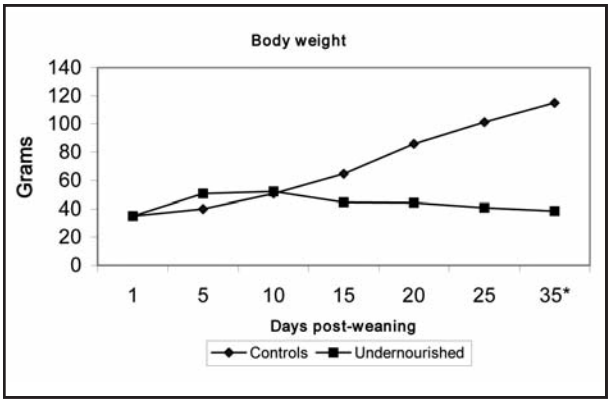

Fig. 2: Body weight in experimental and control groups. Student's t test: p<0.05.

projected image of the radiographs (X7 magnification) in order to perform the cephalometric measurements (Fig. 1). The projected image of the wire served as a scale and the measurements were calibrated according to the image of the standard length of wire. The relationship between molar mesiodistal width and mandibular arch length was calculated using the ET/RS ratio (Fig. 1, Table 2). The differences in linear mandibular dimensions and in ET/RS ratio between the control and experimental groups were analyzed using Student's t test and Mann Whitney's test respectively.

RESULTS

Body Weight

A slight increase in body weight was observed in both groups throughout the first ten days of the study, after which no further increase was detected in the undernourished group. Body weight was significantly lower in experimental rats as compared to controls at the end of the experimental period (Fig. 2).

Mandibular dimensions

The results of the cephalometric analysis are summarized in Table 3.

The length of the mandible as a whole (B-N), of the condylar process (S-K), and of the angular process

TABLE 2. Variables measured on the lateral roentgenograph of the mandible. The variables were defined between two points or between a point and a line. (According to Kiliaridis S (11).

B-N	Total mandibular length
R-S	Chord of the mandibular canal representing the length of the mandibular corpus
B-R	Length of the incisal process
K-L	Length of the condylar head
S-K	Length of the condylar process
P-N	Length of the angular process
E-T	Total mesio-distal width of the 1st, 2nd, and 3rd molars
O-PN	Angular process to mandibular body vertical relationship. The shortest distance between point O and line PN.
O-RS	Convexity of the angular process. The shortest distance between point O and line RS.
C-D	Height of the lower alveolar process

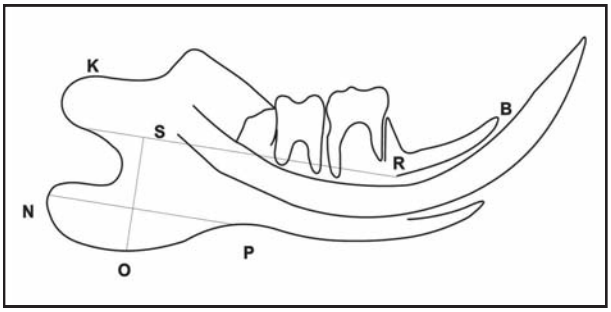

Fig. 3: Variables measured on the lateral roentgenograph of the mandible modified by treatment. B-N: Total mandibular length. S-K: Length of the condylar process. P-N: Length of the angular process. O-PN: Angular process to mandibular body vertical relationship. The shortest distance (perpendicular) between point O and line PN. O-RS: Convexity of the angular process. The shortest distance (perpendicular) between point O and line RS.

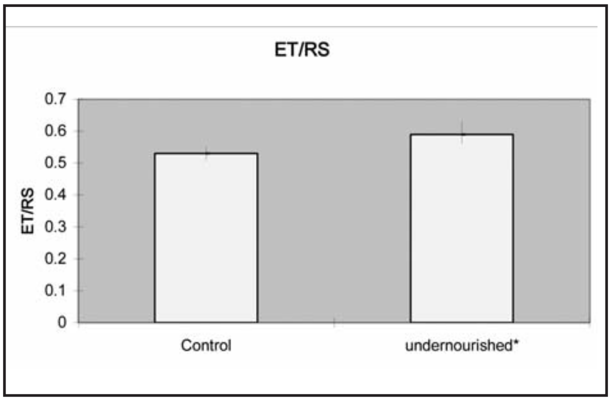

Fig. 4: The ratio between the molar mesiodistal width and the chord of the mandibular canal (representing the length of the mandibular corpus) (Mean and SD)
p<0.05. Mann Whitney's Test.

TABLE 3. Cephalometric determinations

	Controls	Undernourished	Student's Test
B-N	108±3	99±3	P<0.05
R-S	62±2	59±4	NS
B-R	27±1	27±1	NS
K-L	7±1	6±2	NS
S-K	21±2	17±3	P<0.05
P-N	44±7	34±2	P<0.05
E-T	32±2	33±2	NS
O-PN	9±1	7±2	P<0.05
O-RS	33±1	27±2	P<0.05
C-D	10±1	9±1	NS

X±SD.
Measurements in millimeters
+Student's t test

(P-N), was significantly lower in the undernourished group. Significant differences were also observed between groups when comparing angular process to mandibular body vertical relationship (O-PN), convexity of the angular process (O-RS) (Fig. 3), and total molar mesiodistal width relative to mandibular canal chord length (ET/RS). The latter values corresponding to the control and experimental groups were 0.53±0.02 and 0.59±0.04 respectively ($p<0.05$) (Fig. 4). No differences were observed in height of the lower alveolar process between the control and experimental groups.

DISCUSSION

The present study shows that protein malnutrition in growing rats with different masticatory muscle capacities alters the length of the mandible as a whole (B-N), the condylar process (S-K), the angular process (P-N), the angular process to mandibular body vertical ratio (O-PN) and the convexity of the angular process (O-RS), as well as the ratio between total molar mesiodistal width and mandibular canal chord length (ET/RS). However, the diet used in this study did not affect the height of the lower alveolar process (C-D).

Several authors have evaluated the effect of protein malnutrition during growth on mandibular development and on the biomechanical properties of the mandible (3, 4, 5, 6). A study by Kiliaridis, 1989 (11) showed that the physical consistency of diets does not affect the length of the condylar process. Thus, the difference between groups in the physical consistency of the diet would not bias the results and conclusions of the present study. To date, there are no studies on the effect of nutritional deficiencies on the direction of mandibular displacement during facial development and on the ratio between the space available in the arch and the space required for alignment of the teeth without crowding.

According to the results obtained in the present study, malnutrition induced by a soft diet lacking protein affected total mandibular length (B-N). However, no significant differences in mandibular conduct chord length (R-S) and in incisal process length (B-R) were observed between groups, showing that the diet failed to induce alterations in the frontal aspect of the mandible. Therefore, the differences in B-N must be attributed to impaired development of the angular process (P-N). It is possible that the differences in values corresponding to the frontal aspect of the mandible failed to reach statistical significance due to the presence of continuously growing incisors, which were probably not affected by the diet. The mandibular units that exhibited a decrease in size compared to controls are structures that grow and develop by endochondral ossification. It is well documented that this type of ossification is particularly sensitive to a number of nutritional deficiencies (13, 14).

The condyle is the main growth center of the mandible (15, 16) and plays an essential role in the development of the facial type. It is well documented that during normal facial growth, the mandibular symphysis moves down and forward with respect to the other facial structures along Rickett's facial axis (1, 15). This movement is the result of vertical and saggital growth vectors. But it is the vertical growth vector that has a crucial effect on the saggital (anteroposterior) growth direction of the mandibular symphysis. This growth pattern results from the descent of the glenoid fossae and, essentially, from the increase in condyle length (15, 16). The development of both these components compensates simultaneously for the vertical growth of the upper jaw and the upper alveolar process, and of the lower alveolar process (15).

According to the results obtained in this study, it could be posited that the lack of development of the condylar process together with adequate development of the lower alveolar process in undernourished subjects may alter the normal pattern of facial growth. The significant decrease in the size of the condylar process together with the decrease in muscle strength caused by undernutrition (8, 9, 10) might favor the loss of vertical control. In this context, the spatial position of the mandible with respect to the base of the skull would be altered, resulting in ideal conditions for a growth pattern characterized by a downward and backward displacement of the mandibular symphysis and a posterior rotation of the mandible. This situation could result in a dolichofacial growth pattern. This type of alteration in the growth pattern caused by protein undernutrition could also cause an increase in the angle to the base of the skull and a downward inclination of the occlusal plane. Under these conditions it would be plausible to posit that the lower incisors would drift upward and forward to compensate the posterior rotation of the mandible, also determining a marked occlusal curve. In addition, given our finding that the lower alveolar process suffered no alterations, it is likely that the lack of condylar growth would result in premature contact, so that an anterior open bite could be a frequent finding in this type of nutrition deficiency.

Furthermore, the significant differences described herein in the angular process (P-N), the angular process to mandibular body vertical relationship (O-PN) and the convexity of the angular process (O-RS), reveal a lack of development of the angular process. It is well known that the lack of development of the angular process clinically involves an increase in the value of the gonial angle. These conditions lead to the logical inclination of the occlusal plane associated to a tendency to posterior displacement of the mandibular symphysis. This pattern of mandible development is characteristic of individuals with a dolichofacial growth pattern (15).

In agreement with other reports (3-6), the results obtained in this study show a decrease in BN values, indicating an alteration in the anteroposterior growth of the mandible. Given that the mesiodistal length of the molar crowns (E-T) showed no alterations, since they were fully developed by the onset of the experiment, it would be reasonable to predict that this situation might result in a discrepancy between the space required for proper alignment of the teeth, without overcrowding, and the space available in the mandibular arch. This relationship was analyzed considering the ratio of the total mesiodistal length from the first to the third molar, as an indicator of the required space, to the mandibular canal chord length, as an indicator of the space available in the arch. Although no significant differences were observed between the control and the experimental groups when comparing both parameters, the ratio between these was found to be significantly higher in the undernourished group. Despite both groups showing similar mesiodistal molar size, the fact that the undernourished group had less space available in the mandibular arch would account for the higher ratio. Given that protein undernutrition does not affect the length of the mandibular corpus (11), it is improbable that dental crowding should occur in the rat. However, it is probable that clinically mesiodistal molar size-arch length discrepancies should result. Thus, overcrowding could be a frequent finding in this type of nutritional deficiency. However extrapolation of these results to humans should be done with caution.

Mandibular canal chord length was used as a parameter of mandibular corpus growth based on reports in the literature showing its suitability for evaluation of alterations in mandibular dimensions and shape (12).

The public health authorities have failed to pay serious attention to the alterations caused to oral health by nutritional deficiencies, specifically from the orthodontic point of view. It is therefore essential to conduct controlled studies to further investigate the dentoskeletal alterations caused by malnutrition.

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