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Radiographic study of mandibular alterations resulting from a ketogenic diet in growing rats. Its orthodontic significance

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ABSTRACT

Aim: *This study analyzes the development of mandibular condyles and its likely impact on the direction of mandibular displacement during maxillofacial development in a ketogenic diet model in growing rats.* ***Materials and Method:*** *Twenty male Wistar rats, weaned at 21 days, were assigned to one of the following groups: control (fed a regular hard diet ad libitum) and experimental (fed a ketogenic hard diet, ad libitum). After 30 days of diet, ketonemia was determined and animals were euthanized. The mandibles were resected, hemisected at the symphysis and fixed in 10% formalin. Remaining soft tissues were removed. Metallic landmarks were placed in the mandibular foramens of one hemimandible of each rat. The hemimandibles were radiographed and cephalometric study was performed on paper tracings of the projected image of the radiographs. Data obtained were analyzed with Student's "t" Test.* ***Results:*** *Food consumed was lower in the experimental group. Body weight was 179±1 g in controls, and 128±11 g in the experimental group, $p<0.05$. Ketonemia was 0.29±0.042mmol/l in controls and 1.89±0.7 mmol/l in the experimental group, $p<0.05$. Condylar process length and angular process convexity were significantly lower in the experimental group.* ***Conclusions:*** *These results suggest that the ketogenic diet alters the development of the condylar process and the angle of the mandible in rats, promoting a dolichofacial growth pattern.*

Keywords: *ketone bodies - bone development - mandible - craniofacial development.*

Estudio radiográfico de alteraciones mandibulares resultantes de una dieta cetogénica en ratas en crecimiento. Su importancia ortodóncica

RESUMEN

Objetivo: *Este trabajo experimental analiza el desarrollo del cóndilo mandibular, y su posible impacto en la dirección del desplazamiento de la mandibula en el desarrollo maxilofacial en un modelo de dieta cetogénica en ratas en crecimiento.* ***Materiales y Método:*** *Veinte ratas Wistar macho destetadas a los veintiún días de edad se asignaron a dos grupos: control (dieta normal dura ad libitum) y experimental (dieta cetogénica dura ad libitum). Tras 30 días de dieta, se determinó la cetonemia y se sacrificaron los animales. Luego de resecar las mandíbulas, se separaron en la sínfisis, se fijaron en formol y se eliminaron tejidos blandos. Referencias metálicas fueron colocadas en los orificios mandibulares y a continuación se radiografiaron. El estudio cefalométrico se realizó en trazados de la imagen proyectada de las radiografías. Los datos se analizaron con la prueba "t" de Student.* ***Resultados:*** *El consumo de alimentos fue menor en el grupo experimental. El peso corporal fue en controles 179±1 g y 128±11 g en el grupo experimental. La cetonemia fue significativamente mayor en el grupo experimental (0,29 ± 0,042 vs. 1,89 ± 0,7 mmol/l). La longitud del proceso condilar y la convexidad del proceso angular fueron significativamente menores en el grupo experimental.* ***Conclusiones:*** *Estos resultados sugieren que la dieta cetogénica altera el desarrollo del proceso condilar y del ángulo mandibular en ratas, promoviendo un patrón de crecimiento dolicofacial.*

Palabras clave: *cuerpos cetónicos - desarrollo óseo - mandíbula - desarrollo cráneo facial.*

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INTRODUCTION

In humans[1] and rodents[2], it is well established that the anterior portion of Meckel's cartilage initially serves as a template for intramembranous ossification of the mandible, and is then completely resorbed. However, the posterior region of the condylar processes is made of accessory cartilage and in humans, begins endochondral ossification around the 20th week of development. This condylar cartilage is the main mandibular center of growth by endochondral ossification until approximately twenty years of age, and plays a key role in maxillofacial development[1].

Craniofacial development is a highly complex process in which the movement of the mandibular symphysis in relation to the rest of the face is of fundamental importance. It is well documented that in normal conditions, the gnation moves downward and forward along the Ricketts facial axis[3]. This type of growth axis is the result of vertical and sagittal displacement of the mandible[3], whose normal development is assumed to be a condition for orthognathic-type facial development.

Bones may be vulnerable to genetic and environmental factors. While genetic factors determine the shape and size of the head, face and dental arches[4], craniofacial development is also affected by external factors during growth. Studies have reported that certain habits, trauma and premature extraction of deciduous teeth alter the normal growth direction and reduce arch length, causing crowding[5].

Additionally, nutritional alterations that may occur during growth can affect bone development, causing marked variations in bone shape and size[6,7]. "Atkins-style" diets, characterized by low-carbohydrate and high-fat content (LC–HF), are usually promoted for weight loss. These diets encourage the intake of fat and protein, while limiting carbohydrates[8]. Ketogenic diet results from further restricting the protein content of LC–HF diets, and is an established treatment for epilepsy in children[9,10]. Although its efficacy in seizure control has been widely reported in the international literature, ketogenic diets have been reported to have several unfavorable metabolic effects, such as increased adiposity, glucose intolerance, higher serum leptin concentrations, and concurrent lean body mass loss in human and animal studies[11–15]. Additionally, diets with an altered relative abundance of fat, protein and carbohydrates affect bone metabolism, altering the skeletal system. These alterations could include disturbances in endochondral ossification[8], as well as loss of bone mineral density, bone volume, trabecular number, and biomechanical properties in long bones[16].However, the effects of ketogenic diet on growth, shape and displacement of the mandible during maxillofacial development have not yet been evaluated.

When well-defined ketogenic low-carbohydrate, high-fat (LC–HF) diets are used to treat epilepsy in growing children[9,10], it is evident that the mandible develops under the negative influence of this nutritional alteration. Considering the effects of the ketogenic diet on long bones, we hypothesize that this diet could impact mandibular growth and maxillofacial development.

Given that clinical trials are restricted, rats are widely used as experimental models, despite their many morphological and functional differences with humans. Rats can perform free lateral and antero-posterior movements of the mandible due to their diet. Consequently, their mandibular body develops in an anteroposterior direction and has a flat glenoid cavity that differs from the human glenoid cavity, which is deeply shaped by opening and closing movements. These mandibular dynamics result in vertical facial development[17].

Based on the above, the aim of the present experimental work is to assess the influence of a ketogenic diet on condylar process length and angular process convexity through radiographic evaluation.

MATERIALS AND METHOD

Animals

Twenty male Wistar rats, weaned at 21 days, were housed individually with water available ad libitum. Room temperature was maintained at 25ºC with a 12:12 h light-dark cycle.

The experimental procedures were carried out in accordance with The National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NHI publication 85-23 Rev. 1985). The ethics committee of the School of Dentistry of the National University of Tucuman approved the protocol according to RES - ODO - DCHCD - 13765 / 2025

Experimental design

The animals were assigned to one of the following groups: control group (n=10), fed a regular hard diet ad libitum, and experimental group (n=10), fed a ketogenic hard diet ad libitum (Table 1)[18]. Animal body weights and food consumed were recorded throughout the experimental period. Animals were euthanized after 30 days of consuming the diet, following international protocols. Once sedated with an intraperitoneal injection of 0.25 ml of a 5% ketamine solution (Holliday-Scott SA) at a dose of 40 mg/kg body weight, and 0.5 ml of a 1% acepromazine solution (Acedan, Holliday-Scott SA) at a dose of 160 mg/kg body weight, euthanasia was performed with an IP injection of 0.20 ml of 4% sodium pentobarbital and 0.5% sodium diphenylhydantoin (Euthanyle, Brouwer SA) at a dose of 160 mg/kg body weight. The mandibles were then 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[19] were placed in the mandibular foramen of one hemimandible of each rat (Fig. 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, with focus to film distance 40 cm.

Table 1. Comparison between standard diet and ketogenic diet

Projected (per 100 g)	Standard diet	Ketogenic diet
Energy	1338 kJ	1748 kJ
Protein	14.5 g	6.1 g
Fat	4 g	37.1 g
Carbohydrates	55.5 g	3.1 g
Dietary Fiber	4.5 g	27.5 g
Natrium	130 mg	144 mg
Calcium	720 mg	117 mg
Phosphorus	600 mg	81 mg
Vitamin D	2.5 µg	0.03 µg

Displayed nutrients of ketogenic diet is less than 100g due to the loss in manufacture[18]

Biochemical determinations

The state of ketosis was verified at the end of the experiment, by visualizing ketonemia determined by colorimetric methods in blood samples obtained by cutting tail veins.

Radiographic determinations

Reference points were marked on paper tracings of the projected image of the radiographs (X7 magnification) in order to perform the radiographic measurements (Fig. 1). Radiographic reference points identifications, tracings and measurements were conducted by the same author. Linear mandibular dimensions measured on the lateral roentgenograph of the mandible are shown in Table 2[19]. 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 and expressed in millimeters.

Statistical Analysis

Data were analyzed using SPSS software for Windows (version 22.0, IBM Corp, Armonk, NY, USA). The normal distribution of continuous variables was assessed using the Shapiro-Wilk test. Student's "*t*" test was used to compare linear mandibular dimensions between control and experimental groups. Differences were considered significant with $p<0.05$.

RESULTS

Body weight and food consumed

Ketogenic diet led to a statistically significant weight reduction at the end of the experimental period (128±11gr) compared to controls (179±1 g) $p<0.05$.

Total food consumed per rat was: Control group 745 g/rat vs. Experimental group 335g/rat.

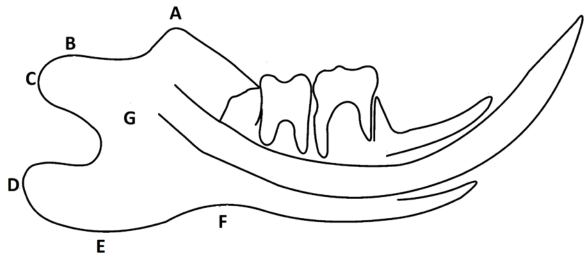

Fig. 1: Reference points defined on the lateral roentgenograph of the mandible, according to Kiliaridis S[19]. A: The most superior point of coronoid process. B: Fovea pterigoidea. C: The most posterior point of the condylar process. D: The most posterior point of the angular process of the mandible. E: The most inferior point of the lower border of the angular process F: The deepest point of the inferior border of the mandible. G: Position of markers in mandibular foramen.

Table 2. Variables measured on the lateral roentgenograph of the mandible.

Code	Description
A-G	Length of the coronoid process.
B-C	Length of the condylar head.
C-G	Length of the condylar process.
D-F	Length of the angular process.
E-DF	Convexity of the angular process. The shortest distance between point E and line DF.

The variables were defined between two points or between a point and a line. (According to Kiliaridis)[19].

Biochemical determinations

Ketonemia was significantly higher in the experimental group. Control group:0.29±0.042 mmol/l vs. Experimental: 1.89±0.7 mmol/l $p<0.05$.

Radiographic determinations

The cephalometric study showed that the condylar process and the convexity of the angular process were significantly lower in the experimental group (Table 3).

DISCUSSION

This study shows that ketogenic diet alters condylar process length (C-G) and angular process convexity (E-DF) in mandibles of growing rats. This is the first study to report mandibular disorders with this diet.

Ketogenic diet negatively influences longitudinal long bone growth in patients with intractable epilepsy[6,20,21]. In this context, it should be noted that histomorphometric studies have reported that this type of diet alters endochondral ossification in children[8,14,16]. Against this background, the growth of the mandibular condylar process that develops through endochondral ossification would also be affected, which would also have a negative impact on normal maxillofacial development.

In rats, the consistency of the diet affects mandibular growth[22], so it is important to emphasize that in the present study, both the control and the ketogenic diets were administered in the form of hard pellets. Although there is a publication[23] reporting that the ketogenic diet in mice does not induce any difference in muscle contraction strength; it should be noted that it was not performed on masticatory muscles. Thus, it is unlikely that the alterations observed in the present study are due to mechanical stimuli, to which the mandible is very sensitive.

Table 3. Radiographic determinations

	Control: Standard Diet	Experimental: Ketogenic Diet	p value
A-G	4.5 ±0.7	4.8 ±0.7	> 0.05
B-C	1.8±0.1	1.6±0.6	> 0.05
C-G	5.2±0.1	4.6±0.4	< 0.05*
D-F	8.8±1	7.5±0.6	> 0.05
E-DF	6.9±0.02	5.6±0.3	< 0.05*

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

It has been clearly demonstrated that, in an orthognathic growth pattern, the descent of the glenoid fossae and growth of the condylar process counterbalance the total vertical downward movement of the upper jaw and upward movement of the lower alveolar process[3, 24]. Clearly, if the vertical development of the maxilla and inferior alveolar processes is less than that of the glenoid fossae and condyles, the mandibular symphysis will predominantly move forward[3, 24]. However, if the vertical development of the maxilla and alveolar processes exceeds the contribution of the condyles, the symphysis will move exclusively downwards, thereby opening up the Rickets facial axis[3, 24].

According to the results obtained in this study, the lack of development of the condylar process in animals fed on a ketogenic diet could alter the normal pattern of facial growth. A significant decrease in the size of the condylar process could lead to a loss of vertical control. This would alter the spatial position of the mandible in relation to the base of the skull, creating 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 could result in dolichofacial growth. Such an alteration to the growth pattern caused by a ketogenic diet could also result in an increased 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 upwards and forwards in order to compensate for the posterior rotation of the mandible, thereby determining a pronounced occlusal curve. On the other hand, the lack of condylar growth likely resulted in premature contact in the molar area. Therefore, an anterior open bite may be a consequence of these nutritional alterations. Furthermore, the significant differences in convexity of the angular process (E-DF) described herein reveal a lack of angular process development. It is well known that a lack of angular process development clinically results in an increased gonial angle. These conditions lead to the occlusal plane inclining logically towards posterior displacement of the mandibular symphysis. This pattern of mandibular development is characteristic of individuals with dolichofacial growth[3]. However, extrapolation of these results to humans should be done with caution.

The results of this study suggest that the ketogenic diet can alter mandibular and angular processes, affecting maxillofacial development, a condition that could be exacerbated if it coexists with oral habits or parafunctions. As the ketogenic diet is effective for controlling seizures in children with epilepsy, it cannot be discontinued. In this context, it is recommended that patients consult an orthodontist with the aim of using interceptive orthodontics to prevent or minimize the negative effects of the ketogenic diet on facial development.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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