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Changes in the Craniomaxillofacial Growth and Development of Sprague Dawley Rats Subjected to Permanent Experimental Unilateral Nasal Obstruction

Cambios en el Crecimiento y Desarrollo Cráneo Maxilo Facial de Ratas Sprague Dawley Sometidas a Obstrucción Nasal Unilateral Experimental Permanente

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SUMMARY: Breathing is considered a vital function dependent on factors such as adequate permeability of the nasal route, which is linked to physiological functions, intellectual processes, and craniofacial growth. The aim of this study was to determine the changes in the craniomaxillofacial growth and bone development of Sprague Dawley rats subjected to permanent experimental unilateral nasal obstruction. Twenty-four newborn rats were used, randomized, and divided into experimental and control groups. The right nostril was obstructed, and weight, length, and Lee's index measurements were recorded at 8 and 16 weeks. Craniomandibular x-rays were taken of each animal, obtaining linear neuro- and viscerocranial measurements. Then, a biochemical analysis was performed to measure the alkaline phosphatase concentration. The results were analyzed in the SPSS software, performing a descriptive analysis, using a t-test for independent samples, comparing basal, cephalometric, and biochemical characteristics between the control and experimental groups, considering a significance range of 5%. When comparing the experimental and control groups, the variables length, weight, and Lee's index presented no significant differences. In the x-ray analysis, at 8 weeks, the Co-L1 and Co-Mn measurements were reduced, whereas the Ba-So increased, with significant differences. At 16 weeks, the L1-O, Po-Ba, and E-Mu measurements decreased; however, Co-Gn registered a greater value with significant differences. The alkaline phosphatase levels fell significantly at week 16 in the experimental group. In conclusion, the reduction of permanent nasal respiratory flow is related to modifications in facial growth at 8 and 16 weeks and to the reduction of alkaline phosphatases at 16 weeks.

KEY WORDS: Nasal obstruction; Maxillofacial growth; Craniofacial morphology; Rats.

INTRODUCTION

The respiratory function is vital and depends on such factors as the adequate permeability of the nasal route (Veron *et al*., 2016), participating in the flow of oxygen, and carbon dioxide, the olfactory function (Alves & Cândido, 2016), intellectual processes (Azagra-Calero *et al*., 2012; Urschitz *et al*., 2003), and in craniofacial growth (Padzys *et al*., 2012).

The air volume that passes through the nose and nasopharynx is limited by the shape and diameter of these structures. Several factors can obstruct these zones and modify the nasal breathing mode at rest, causing a predominantly oral respiratory mode. Common causes are pharyngeal tonsil hypertrophy, palatine tonsil hypertrophy, congenital nasal deformities, nasal polyps, or nasal trauma (Schlenker *et al*., 2000), alteration in the maxillary and mandibular position (D'Ascanio *et al*., 2010; Li *et al*., 2022), as well as tumor lesions, and allergic or non-allergic inflammatory pathologies (Sato *et al*., 2018).

Craniofacial growth occurs in relation to functional aspects, so that the genotype and phenotype take part in their development. It is possible to confirm that no craniofacial component develops independently; Sato *et al*. (2018) propose a strong link between facial growth and functional activity in

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the craniocervical region. In the same way, respiratory conditions are related to facial characteristics (Cillo *et al*., 2012), and airway volume is related to facial morphology (Ravelo *et al*., 2020; Ravelo *et al*., 2021).

Studies with rats subjected to intermittent hypoxia have reported alterations in craniomaxillofacial growth, with clockwise mandibular rotation and dentomaxillary anomalies (Mattar *et al*., 2004; Harari *et al*., 2010), impacting in addition mandibular bone mineral density (Oishi *et al*., 2016). Hong *et al*. (2021) studied the effects of intermittent hypoxia in adolescent rats, concluding that this condition increases bone mineral density in the condylar head of the mandibular process, so it is possible to observe a relation between airflow alteration and alterations in craniofacial growth. The influence of the permanently reduced respiratory condition has not yet been defined.

The model in rats is interesting, as in this species the maxillary bones experience high growth and development from prepuberty to the young adult period (Pallav, 2013). The aim of this study was to determine the changes in craniomaxillofacial bone growth and development in Sprague Dawley rats subjected to permanent experimental unilateral nasal obstruction.

MATERIAL AND METHOD

Animal model and analysis design. Twenty-four newborn male Sprague Dawley rats (8 days old) were randomized and divided into a control group (CG) and an experimental group (EG). The EG (n=12) was subjected to nasal obstruction (NO) by means of a simple suture of the right nostril; the monitoring of the rat and the area of intervention was daily during the first week and then every 3 days to confirm the permanence of the NO.

The follow-up was done with measurements at weeks 8 and 16, so 4 working groups were included:

1. Experimental group (nasal obstruction) 8 weeks (EG-8),
2. Control group, without NO 8 weeks (CG-8),
3. Experimental group (nasal obstruction) 16 weeks (EG-16),
4. Control group, without NO 16 weeks (CG-16),

During the experimental period, the rats were kept for 16 weeks in the vivarium of the Center for Excellence in Morphological and Surgical Studies (CEMyQ) at the Universidad de La Frontera, Temuco, Chile, in individual cages at 22±2°C and 50-70% humidity and a 12 h light/dark cycle (8:00 a.m.– 8:00 p.m. /8:00 p.m. – 8:00 a.m.). They were given a standard laboratory diet (AIN-93M) and water ad libitum. The study considered the guidelines in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). This study was approved by the Universidad de La Frontera's Scientific Ethics Committee (Nº 025/2016).

The animals' body weight was measured weekly on an analytical balance (Radwag, WTB2000, Poland). Lee's index was calculated (De Campos *et al*., 2007), where a value equal to or less than 0.3 was considered rats with normal weight and a value greater than 0.3 rats with obesity (Roa & del Sol, 2021).

X-ray analysis. At the end of the experimental period (8 and 16 weeks), latero-lateral and cranio-ventral craniomandibular X-rays were taken of each animal with an X-ray machine after anesthesia with ketamine-xylazine (80/10 mg/Kg respectively). The linear measurements L1-O, L2-O, L1-L2, X1-X2, E-U, Go-Mn, M1-L1, Co-L1, Co-Gn, Co-Mn, Po-E, Ba-E, So-E, Ba-So, Po-Ba, E-N and E-Mu were taken on the x-rays at the neuro- and viscerocranial level, as shown in Fig. 1 and described in Table I and Table II. The measurements were taken by an observer with a two-week interval between them. Then, an intraclass correlation study was done to standardize the measurement process, resulting in p=0.8.

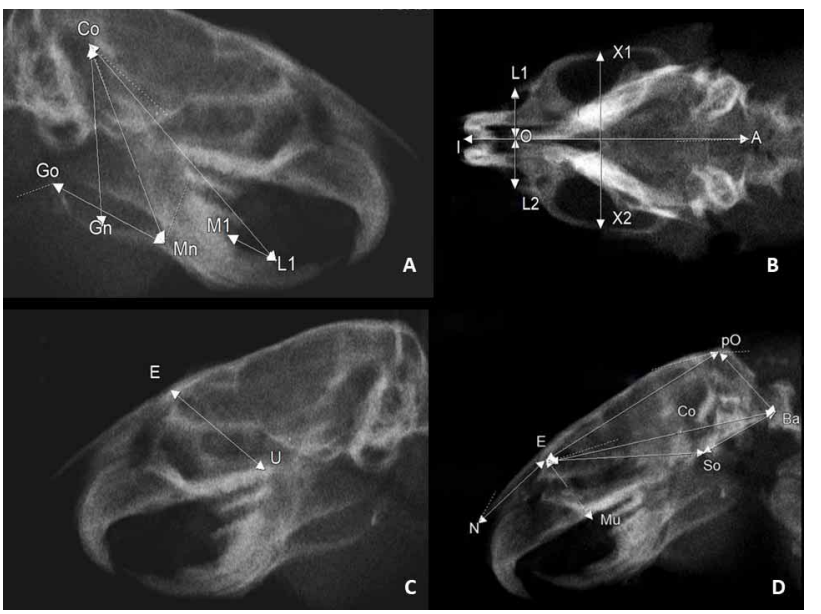

Fig. 1. Cephalometric measurements of Sprague Dawley rats. (A) Viscerocranium measurements, (B) Superior measurements, (C) Total height of the nasomaxillary complex, (D) Neurocranium measurements.

Table I. Definitions of cephalometric landmarks in Sprague Dawley rats.

Cephalometric Landmark	Definition
N	The most anterior point on the nasal bone.
E	The intersection of the frontal bone and floor of the anterior cranial fossa.
U	Intersection between the maxillary sinus and the distal surface of the third superior molar.
Po	The most posterior and superior point on the skull.
Ba	The most posterior and inferior point on the occipital condyle.
So	The intersection of the most anterior tympanic bulla and the superior margin of the sphenoid bone.
Go	The most posterior point on the mandibular ramus.
Mn	The most concave portion of the concavity on the inferior margin of the mandibular corpus.
Co	The most posterior and superior point on the mandibular condyle.
Gn	The most inferior point of the ramus that lies on a perpendicular bisector of the Go-Mn line.
Mu	Junction of alveolar bone and mesial surface of maxillary first molar.
L1	Most anterior and superior point of the zygomatic process of the right maxilla.
L2	Most anterior and superior point of the zygomatic process of the left maxilla.
X1	Point of intersection between a straight line passing through point P and normal to the median plane.
X2	Point of intersection between a straight line passing through point P and normal to the median plane.
O	Point of intersection of the premaxillary-palatal junction on the A-I line.
A	Most posterior point of the occipital bone.
I	Upper interincisor point.
L1m	Most anterior and superior point of the alveolar bone of the mandibular notch.
M1	Junction of the alveolar bone and the mesial surface of the mandibular first molar.

Table II. Cephalometric measurements of the craniofacial skeleton.

	Cephalometric measurement	Definition
Neurocranium	Po-E	Cranial vault length
	Ba-E	Total cranial base length
	So-E	Anterior cranial base length
	Ba-So	Posterior cranial base length
	Po-Ba	Posterior neurocranium height
Viscerocranium	E-N	Nasal length
	E-Mu	Viscerocranial height
	Co-Gn	Ramus mandibular height
	Co-L1	Total length of the mandibular body
	Co-Mn	Mandibular ramus length
	Go-Mn	Posterior corpus length
	M1-L1	Mandibular body anterior length
Superior measurements	A-I	Total skull length
	L1-O	Mediolateral development of the right maxillary
	L2-O	Mediolateral development of the left maxillary
	L1-L2	Overall development of the two maxillae
	X1-X2	Transverse development of the most posterior region of the middle cranial fossa
	E-U	Total height of the nasomaxillary complex

Biochemical analysis. At the end of the experimental period (8 and 16 weeks), the animals were kept fasting for 6 hours and then euthanized with anesthetic overdoses. A blood sample was immediately extracted by cardiac puncture of the right atrium into a tube with EDTA for the biochemical analysis. The plasma was separated by centrifugation (1200¥ g for 15 minutes) and stored at -80°C. The alkaline phosphatase (ALK) concentration was analyzed in a CM250 Wiener biochemistry analyzer.

Statistical analysis. The results were recorded in a database generated in Microsoft Excel 2016 and analyzed in the SPSS statistics software (IBM SPSS Statistics, v. 21). The descriptive analysis of the variables was expressed as mean + standard deviation (SD). The Shapiro-Wilk test was used to determine the normality of the data. The basal characteristics between the groups were compared using a t-test for independent samples. The cephalometric and biochemical variables were compared between the experimental and control groups, and

a t-test was used for independent samples. The level of statistical significance was set at $p<0.05$.

RESULTS

The experiment was developed without complications. The animals responded adequately to the entire process. At the beginning of each experiment, the animals were evaluated by Lee's index and the length of the animal to obtain the initial record. The comparison of the control and experimental groups at 8 and 16 weeks in length and weight presented no significant differences, so the nasal obstruction did not generate significant changes with these variables (Table III).

When analyzing the measurements obtained from the x-rays comparing the experimental and control groups, a reduction was noted in the experimental group of Co-L1 (p=0.048), and a reduction of Co-MN (p=0.046) with significant differences; likewise, the Ba-So length presented a significantly greater measurement for EG-8 (p=0.029) (Table IV)

In the evaluation at 16 weeks (Table III), there was a significant reduction in the experimental group of L1-O (p=0.030), Po-Ba (p=0.026), and E-Mu (p=0.009). In addition, Co-Gn registered a greater value in EG-16 than in CG-16, with statistically significant differences (p=0.007).

The biochemical analysis of the ALK ended with no significant differences observed in the eighth week (p=0.641); however, at week 16, the ALK levels dropped significantly in the experimental group (p=0.011), as illustrated in Table V.

Table III. Length, weight, and Lee's index in Sprague Dawley rats of the control group and experimental group at the beginning of the study.

Variable	GC-8	GE-8	p	GC-16	GE-16	p
Length (cm)	18.85 ± 0.67	19.48 ± 0.61	0.143	25.10 ± 1.08	25.25 ± 0.61	0.779
Weight (g)	50.40 ± 3.22	53.82 ± 6.87	0.303	50.90 ± 5.27	58.67 ± 7.04	0.074
Lee's index	0.20 ± 0.000	0.19 ± 0.01	0.463	0.20 ± 0.01	0.19 ± 0.01	0.124

* Differences were considered statistically significant when $p<0.05$.

Table IV. Cephalometric measures in Sprague Dawley rats in the control and experimental groups at 8 and 16 weeks.

Variable	CG-8	EG-8	p	CG-16	EG-16	p
L1-O	6.17 ± 0.45	6.06 ± 0.38	0.661	6.81± 0.73	6.00 ± 0.25	0.030*
L2-O	6.32 ± 0.39	6.36 ± 0.44	0.886	7.27± 0.58	6.85 ± 0.28	0.151
L1-L2	12.50 ± 0.54	12.42 ± 0.78	0.847	14.08 ± 1.30	12.85 ± 0.42	0.055
X1-X2	20.67 ± 4.86	22.64 ± 0.18	0.395	24.49 ± 0.74	24.07 ± 0.19	0.213
E-U	12.83 ± 0.56	13.13 ± 0.38	0.339	13.71 ± 0.91	14.29 ± 0.57	0.226
Go-Mn	12.02 ± 1.28	12.09 ± 2.99	0.959	13.51 ± 1.10	14.06 ± 0.77	0.354
M1-L1	6.14 ± 0.37	5.56 ± 0.71	0.116	7.41 ± 0.54	7.85 ± 0.57	0.226
Co-L1	27.97 ± 2.04	25.62 ± 1.13	0.048*	29.85 ± 1.32	31.10 ± 0.93	0.099
Co-Gn	15.44 ± 2.73	12.38 ± 2.11	0.071	13.92 ± 0.85	16.65 ± 1.57	0.007*
Co-Mn	18.88 ± 2.09	15.65 ± 2.54	0.046*	18.98 ± 1.25	20.41 ± 1.27	0.094
Po-E	27.04 ± 1.44	27.58 ± 1.58	0.571	27.27 ± 1.32	28.91 ± 1.69	0.112
Ba-E	28.47 ± 1.63	30.16 ± 2.22	0.178	30.98 ± 1.12	31.60 ± 1.27	0.417
So-E	19.32 ± 1.99	20.17 ± 1.63	0.465	22.29 ± 2.18	21.78 ± 1.21	0.633
Ba-So	10.23 ± 0.61	11.36 ± 0.84	0.029*	10.40 ± 1.52	11.25 ± 0.67	0.243
Po-Ba	9.97 ± 0.62	11.15 ± 1.34	0.084	11.51 ± 0.47	10.37 ± 0.86	0.026*
E-N	16.43 ± 1.44	14.42 ± 1.91	0.077	18.58 ± 1.71	18.89 ± 1.22	0.734
E-Mu	10.97 ± 0.96	11.20 ± 1.08	0.710	12.96 ± 0.49	11.95 ± 0.51	0.009*

*Differences were considered statistically significant when $p<0.05$.

Table V. ALK levels in Sprague Dawley rats in the control and experimental groups at 8 and 16 weeks.

Variable	CG8	EG8	p	CG16	EG16	p
ALK	602.14 ± 351.38	694.80 ± 267.10	0.641	894.08 ± 276.89	407.00 ± 226.30	0.011*

*Differences were considered statistically significant when $p<0.05$.

DISCUSSION

Some markers have been used in the bone remodeling analysis. The ALK enzyme is a bone marker that determines osteoblastic activity to maintain the density and integrity of the bone matrix (Barco *et al*., 2012). Our results show that the ALK enzyme was significantly reduced at 16 weeks of sustained unilateral nasal obstruction; this phenomenon did not appear at 8 weeks. Our results agree with previous reports by various groups (Wolford *et al*, 1987; Alemáan *et al*., 1998), which show the ALK enzyme with significantly high values in rapidly growing animals and then gradually decreasing with aging.

This may be associated with the inherent growth and aging of the model animal. Nevertheless, in the group of rats with nasal obstruction and, therefore, reduced airflow, there was a significant reduction of ALK, which may link respiratory flow to ALK deficiency. In humans, the reductions in the serum activity of ALK are related to changes in the bone mineral metabolism and clinical manifestations such as the skeletal and dental hypomineralization characteristic of hypophosphatasia (Millán *et al*., 2016; Martos-Moreno, 2018). The reduction in the amount of ALK may be linked to differences in the craniomaxillofacial bone growth in rats in the same group, which was confirmed by x-rays.

Bone modeling and remodeling affect local mechanical loads at the tissue level (Crespo & Gomar, 2000), so the neuromuscular activity during breathing may also be associated with growth alterations. The oral respiratory mode appears as a compensatory mechanism against nasal obstruction, which requires modification in the position of the mandible and tongue to achieve the entry of air via the oral cavity. The obstruction of the upper airway affects the mandibular opening reflex (Funaki *et al*., 2014), since hypoxia causes the inhibition of the postsynaptic components in the vestibular nuclei, which are connected to the spinal nuclei of the trigeminal nerve that takes part in the mandibular opening reflex (Pinganaud *et al*., 1999); in addition, it is associated with changes in the craniofacial bone growth of rats (Padzys *et al*., 2012)

In this study, no modifications were reported in neurocranial measurements, a phenomenon that agrees with the report by Oishi *et al*. (2016). Nasal obstruction can influence the viscerocranial measurements, such as Co-L1 and Co-Mn, which showed a reduction in EG-8.

Oishi *et al*. (2016) reported similar results in rats subjected to NO for 3 weeks for the distance Co-L1. The distance Ba-So was greater for EG-8, which may correspond to a compensation mechanism. Another relevant aspect is that the condyle of the mandibular process has a high growth impact on later stages to stimulate facial development so that the maintenance of nasal obstruction might not interfere with the growth and development of the neurocranium, but rather of the viscerocranium.

In EG-16, less mediolateral development of the right maxilla (L1-O) was identified, possibly due to the right nostril being obstructed at the beginning of the experiment. Notably, this difference was statistically significant at 16 weeks and not at 8, which could be related to the effects of the NO on later stages of development. Moreover, in EG-16, the height of the mandibular ramus (Co-Gn) had a higher value, whereas the posterior length of the neurocranium (Po-Ba) and the height of the viscerocranium (E-Mu) demonstrated a lower value than the control group.

Based on our results, we may conclude that the reduction of permanent nasal respiratory flow is related to changes in facial growth at 8 and 16 weeks and to the reduction of ALK analyzed at 16 weeks.

MOYA, M. P.; & OLATE, S. Cambios en el crecimiento y desarrollo cráneo maxilo facial de ratas Sprague Dawley sometidas a obstrucción nasal unilateral experimental permanente. *Int. J. Morphol., 41(4)*:1273-1278, 2023.

RESUMEN: La respiración se considera una función vital, dependiente de factores como la permeabilidad adecuada de la vía nasal, vinculada con funciones fisiológicas, procesos intelectuales y crecimiento cráneofacial. El objetivo de este estudio fue determinar los cambios en el crecimiento y desarrollo óseo cráneo maxilo facial de ratas Sprague Dawley sometidas a obstrucción nasal unilateral experimental permanente. Se utilizaron 24 ratas macho neonatas, randomizadas y divididas en grupo control y experimental. Fue realizada obstrucción nasal de la narina derecha y realizadas mediciones de peso, longitud e índice de Lee a las 8 y 16 semanas. Se efectuaron radiografías cráneomandibulares a cada animal, obteniendo medidas lineales de neuro y viscerocráneo. Posteriormente se realizó análisis bioquímico, para medir la concentración de fosfatasa alcalina. Los resultados fueron analizados en el software SPSS, realizándose análisis descriptivo, empleando prueba T para muestras independientes comparando características basales, cefalométricas y bioquímicas entre los grupos control y experimental, considerando un umbral de significancia de 5 %. Al comparar los grupos control y experimental, las variables longitud, peso e índice de Lee no presentaron diferencias significativas. En el análisis radiográfico, a las 8 semanas, las medidas Co-L1 y Co-Mn presentaron reducción, mientras que Ba-So aumentó, con diferencias significativas. A las 16 semanas, las medidas L1-O, Po-Ba y E-Mu disminuyeron, sin embargo, Co-Gn registró un mayor valor, con diferencias significativas. Los niveles de fosfatasa alcalina disminuyeron significativamente

en la semana 16 en el grupo experimental. En conclusión, la reducción de flujo respiratorio nasal permanente se relaciona con modificaciones del crecimiento facial a las 8 y 16 semanas y con la reducción de ALK en análisis a las 16 semanas.

PALABRAS CLAVE: Obstrucción Nasal; Crecimiento maxilofacial; Morfología cráneofacial; Ratas.

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