Effects of an Obesogenic Diet on Craniofacial Morphology in Rats

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Abstract

To assess the effect of an obesogenic diet on craniofacial morphology in Wistar rats during the preadolescence and young adult stages.

Twenty-one-day-old male Wistar rats were divided into two groups according to feeding: one fed a standard diet (SD) the second a cafeteria diet (CD). Six 38-day-old pre-adolescents (three in SD control group and three in the CD group) and ten 77-day-old young adult biomodels (five in the SD control group and five in the CD group). The weight, length, abdominal circumference, and blood glucose were taken for each rat. At the end of the experiment, dissections were performed to obtain samples of the skull, mandible, and perigonadal fat. Digital morphometry and manual cephalometry were performed on each sample.

The 38-day-old preadolescent rat samples did not present statistically significant differences in the evaluated parameters. 77-day-old young adult biomodels in the CD group presented higher weight ($p \leq 0.01$), length ($p \leq 0.05$), abdominal circumference ($p \leq 0.05$), perigonadal fat ($p \leq 0.005$) compared to the SD group. The CD group presented a greater nasal length ($p \leq 0.03$), narrower nasal width ($p = 0.0001$), greater maxillary length ($p \leq 0.02$), an increase in nasal bone length ($p \leq 0.02$), and a more pronounced lower incisor inclination ($p \leq 0.06$), compared to the SD group. At 49-day-old, an increase in blood glucose was recorded for the CD group, which coincides with the stage of sexual maturation.

In conclusion, administering an obesogenic diet to rats during bone development and growth alters morphology of the craniofacial complex.

Experimental article (J Int Dent Med Res 2021; 14(1): 5-11)

Keywords: Bone and bones, growth and development, mandible, obesity, skull.

Received date: 25 September 2020 **Accept date:** 22 October 2020

Introduction

The global obesity epidemic represents a major clinical and public health crisis.[1] The most recent data from the World Health Organization (WHO) reveals that 13% of the total adult population is classified as obese and 39% overweight. Global trends are not encouraging: some 41 million children under the age of five, and 340 million adolescents between the ages of five and 19, are overweight.[2]

WHO in its latest report shows that obesity in children of both sexes, between five and nine years old, and in adolescents, also of both sexes, between ten and 19 years old, is rapidly increasing. In America, children with obesity reach 22.7% in the United States of America and 21.7% in Argentina. For adolescents, these same countries are on the top of the list with 25.7% y 14.4%, respectively. In Europe, obese children reach 17.8% in Greece and Italy, and for adolescents, the same trends are presented in these countries with 11.7% and 9.8%, respectively. Worldwide, Nauru and Cook Island, both islands of the pacific; prevalence of obesity in children reaches 36.3% and 36.1%, respectively. For adolescents, it reaches 31.7% and 30.3% respectively.[3]

Obesity has been associated with well-known systemic effects such as high blood pressure, diabetes, stroke, digestive diseases, various types of cancer, osteoporosis, and osteoarthritis.[2] Other effects, less well-investigated and less known, are the differential

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growth of the jaws, greater predisposition to generate dental caries and periodontal diseases. Regarding the effects at the bone tissue level, obesity plays a role in both osteoblastogenesis and osteoclastogenesis; where raised levels of the hormones leptin, adiponectin, and some cytokines lead to decreased bone mass.[4]

It has been shown that obese individuals in adolescence present specific morphological changes such as an increase in the skull base,[5-8] in the length of one or both jaws,[5-10], in the length of the mandibular body[6] and the length of the midface.[10]

For this reason, understanding obesity and its effects on craniofacial development present a new challenge for the scientific community, given its potential role in the dysmorphism of facial structures, affecting not only the final phenotype of the face but also the bone structure and respiratory function. Therefore, the objective of this study was to evaluate the craniofacial morphology of the Wistar rat, in the preadolescent and young adult stages, after consuming an obesogenic diet.

Materials and methods

Animal care

This research was approved by the Institutional Review Committee on Ethics for Experimental Animals, 013-017. The biomodels were housed in the Intermediate Laboratory of Preclinical and Animal Research - Bioterium, in the Universidad del Valle, Cali, Colombia, which complies with the requirements of national and international regulations.

Experimental model

Sixteen 21-day-old male Wistar rats (*Rattus, norvergicus, Berkenhout*) were weaned for separation from the mother and singly housed in controlled temperatures (23 ± 1 ° C) and artificially controlled light-dark conditions (illumination from 06:00 hours to 18:00 hours).
Eight specimens were randomly designated as control biomodels, receiving a standard diet (SD). The Remaining eight experimental biomodels were fed a cafeteria diet (CD) characterized by its high-fat content and supplemented with a standard diet. All biomodels had access to water *ad libitum,* both diets where provided from day 21 until euthanasia.

The standard diet consisted of a fixed supply of food pellets (Purina, LabDiet 5010). The processed food diet (cafeteria diet) consisted of administering three randomly selected foods daily which included: brownies (three types), cake (two types), sponge cake (seven types), cheese-flavored corn sticks, canned Vienna sausage, cereal hoops, puffed corn cereal, caramel-coated popcorn, giant fried corn, fried yucca snacks, achira (Andean tuber) snack-bites and breadsticks. Excessive portions were dispensed of both diets with a protocol modified from Tejada et al in 2016.[11]

Weight, length (snout to tail base), and abdominal circumference for each biomodel were recorded weekly. Additionally, glycemic levels were evaluated at the beginning (21 day-old), middle (49 day-old), and endpoints of the experiment (38-day-old and 77-day-old). The biomodels were characterized as follows: six 38-day-old preadolescents (three in SD control group and three in the CD group) and ten 77-day-old young adult biomodels (five in the SD control group and five in the CD group). Figure 1.

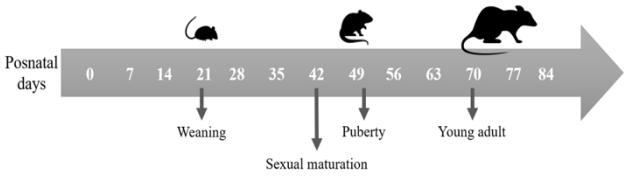

Figure 1. Phases on postnatal days (PND) of a Wistar rat.
These animals are weaned on Day 21, commence sexual maturation around Day 42, reach puberty on Day 50, and become young adults by Day 70 (Sengupta 2013). The highest peak of bone growth occurs between PND 21 and 70.

Once euthanized, the perigonadal fat and the head were obtained. The perigonadal fat mass was weighed. From the head, the skin, eyes, tongue and brain were removed.

They were placed in an oven at 56 ° C for 12 hours and were subsequently treated with the beetle larvae of the Dermestidae family (*Anthrenus verbasci, Coleoptera, Latreille*)[12] for approximately two weeks, after which the jointed bones of the skull and mandible were obtained.

Morphometric parameters

For each sample, four digital images were obtained from three views, (lateral, ventral and dorsal), using a stereo microscope[12] (MEIJI Techno, RZT Stand), connected to a camera (Infinity, reference 3) and an image acquisition software[13,14] (Infinity version 5.0.3.). The images

were processed using the free software Image J (Natural Institutes of Health), the morphometry was based on morphometric points and distances from the studies by Lele et al in 1991 and Richtsmeier et al in 2000, using the EDMA and other morphometric techniques as employed by Fernandes et al in 2008, Yang et al in 2011 and Hichijo et al in 2014.[12-16] All measurements are shown in millimeters (mm). Table 1 and Figure 2.

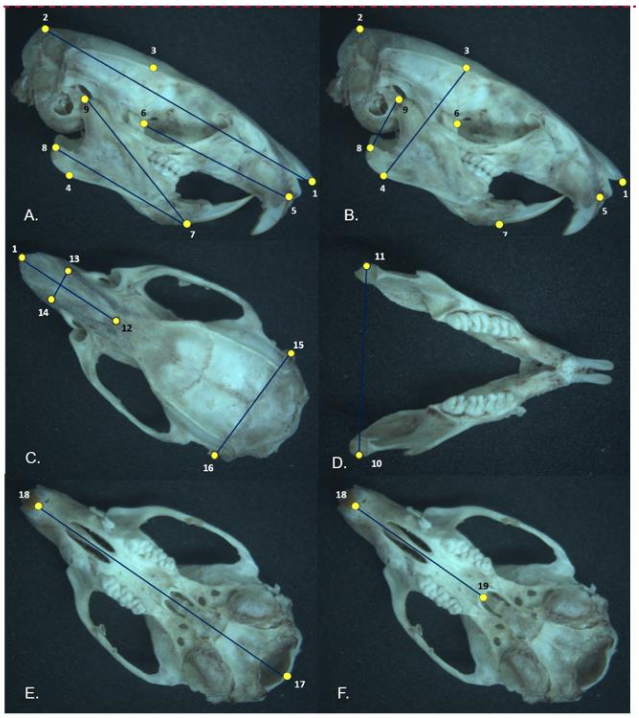

Figure 2. Morphometric distances are shown from different anatomical views.
A. Lateral view of the skull and mandible: 1-2 Maximum length of the neurocranium, 5-6 Maxillary length 1, 7-8 Mandibular corpus length, 7-9 Mandibular length; B. Lateral view of the skull and mandible: 3-4 Skull height, 8-9 Mandibular ramus length; C. Dorsal view of the skull: 1-12 Nasal length, 13-14 Nasal width, 15-16 Skull width; D. Dorsal view of the mandible: 10-11 Mandibular width; E. Ventral view of the skull: 17-18 Skull base length; F. Ventral view of the skull: 18-19 Maxillary length 2. Photographs at 7.5X.

An X-ray was also obtained for each sample, employing equipment used for human radiography (Gendex, Gx-770) and using the same digital periapical film (DIGORA, Optime DXR-50 001). Stable bone positioning was achieved using a parallelometer (DAC) fixed with wax. For manual cephalometry, cephalometric points and distances defined by Engström et al in 1982 and Abbassy et al in 2008, and cephalometric angles defined by Engström et al in 1982 were used.[17-18] All distances were measured in mm and all angles in degrees. Table 2 and Figure 3.

Point	*Description*
N	A point on the nasofrontal suture
A	The most anterior point on the nasal bone
E	The intersection between the frontal bone and the most superior-anterior point of the posterior limit of the ethmoid bone
So	The intersection between the posterior border of the basisphenoid and the tympanic bulla
Pr	The most inferior and anterior point on the alveolar process of the premaxilla
Bu	A point on the premaxilla between jaw bone and the lingual surface of the upper lingual incisors
Iu	The most prominent point between the incisal edges of the upper incisors
Mu	A point on the intersection between the maxillary bone and the mesial surface of the upper first premolar
Ii	The most prominent point between the incisal edges of the lower incisors
Id	The most inferior and anterior point on the alveolar process of the mandible
Mn	A point in the deepest part of the antegonial notch curvature
Ml	A point on the intersection between the mandibular alveolar bone and the mesial surface on the first premolar
Bl	A point on the intersection between the lingual surface of the lower incisors and the most anterior part of the lingual alveolar bone
Co	The most posterior and superior point on the mandibular condyle
Go	The most posterior point on the mandibular ramus

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Go	The most posterior point on the mandibular ramus

Table 2. Cephalometric points.

Statistical Analysis

The data was presented as the mean ± standard deviation. GraphPad Prism Version 6.01 (GraphPad software, San Diego, CA) was used for the statistical analysis. The statistical significance of the experimental observations for animal characterization was determined using one-way ANOVA plus Tukey´s test multiple comparisons test. On the other hand, for morphometry and cephalometry of 77-day-old young adult samples was determined using the Student's t-test and two-way analysis of variance (ANOVA). The level of significance was set at $p \leq 0.05$.

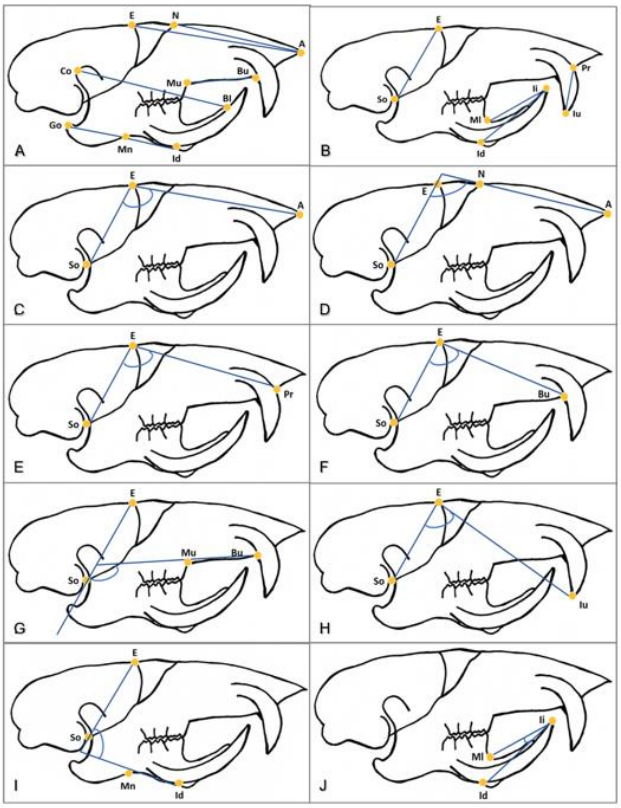

Figure 3. Cephalometric distances and angles.
A. Horizontal cephalometric distances: E-A Anterior skull base length, N-A Nasal bone length, Mu-Bu Palate length, Co-Bl Total mandibular length, Go-Mn Posterior mandibular corpus length, Mn-Id Mandibular corpus length; B. Vertical cephalometric distances: So-E Posterior skull base length, Iu-Pr Erupting upper incisor length, Ml-Ii Lingual lower incisor length, Ii-Id Buccal lower incisor length; C. Cephalometric angle: E-A/So-E Anterior skull base length to posterior skull base length; D. Cephalometric angle: A-N/So-E Nasal bone length to posterior skull base length; E. Cephalometric angle: Pr-E/So-E Viscerocranial length to posterior skull base length 1; F. Cephalometric angle: Bu-E/So-E Viscerocranial length to posterior skull base length 2; G. Cephalometric angle: Mu-Bu/So-E Palate length to neurocranial length; H. Cephalometric angle: E-Iu/So-E Erupting upper incisor length to posterior skull base length; I. Cephalometric angle: Mn-Id/So-E Mandibular corpus length to cranial base length; J. Cephalometric angle: Ml-Ii/Ii-Id Buccal lower incisor length to lingual lower incisor length.

Results

The biomodels fed the cafeteria diet presented significant increases in weight, length, abdominal circumference, and perigonadal fat compared to those fed standard diet. The weight gain percentage was higher compared to the biomodels fed the standard diet.

When comparing weight gain between the two groups from Day 35 of age (p≤0.01), and up to Day 77, those in the CD group were found to have gained more weight (CD: 299.5 ± 11.4 g vs. SD: 270.7 ± 8.0 g; p≤0.01). Those in the CD group had longer body lengths from 28 days of age than the SD group (p≤0.05). Interestingly, the lengths of the biomodels in both groups were similar on day 35 and between Days 42 to 49, a stage that coincides with sexual maturation. However, from Day 56, they differ in length, and by Day 77, a more significant increase in body length is again seen in the CD group compared to the SD group (CD: 20.7 ± 0.4 cm vs. SD: 19.2 ± 0.5 cm; p≤0.05). Analyzing abdominal circumference, it was evident that both 38-day-old preadolescent animals and 77-day-old young adults in the CD group showed a greater increase in this measure compared to the biomodels in the SD group (CD: 19.4 ± 0.1 cm vs. SD: 17.3 ± 0.2 cm; p≤0.05); with the difference between groups being statistically significant for all days of life, bar Day 56. Perigonadal fat presented similar values in both groups for Day 38 (CD: 0.5 ± 0.1 g. vs. SD: 0.6 ± 0.4 g), but this fat was significantly higher in the CD group by Day 77 (CD: 6.4 ± 0.9 g vs. SD: 3.6 ± 0.6 g; p≤0.005). Figure 4.

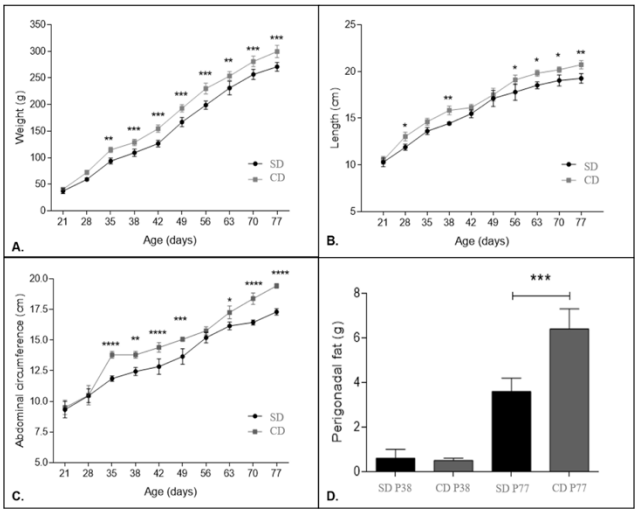

Figure 4. Animal characterization.
A. Weight growth curve; B. Length growth curve: C. Abdominal circumference growth curve. Values represent mean ± standard deviation *p≤0.05 **p≤0.01 ***p≤0.001 ****p≤0.0001. D. Perigonadal fat chart ***p≤0.005.

Glycemic levels were measured at three points during the testing period, each taken following an eight hour fasting period: 21-day-old neonates (SD: 106.7 ± 17.7 mg/dL), 38-day-old preadolescents (SD: 108 ± 23.6 mg/dL vs. CD: 98.7 ± 5.1 mg/dL), 77-day-old young adults in the middle of the experiment with 49 days of age (SD: 104.8 ± 9.4 mg/dL vs. CD: 140.8 ± 12.6 mg/dL), and at the end of the experiment with 77 days of age (SD: 91.2 ± 4.9 mg/dL vs. CD: 102.6

± 5.1 mg/dL).

Morphometry and cephalometry of 38-day-old preadolescents from the SD and CD groups do not show statistically significant differences for the evaluated parameters. In contrast, morphometry showed that 77-day-old young adult biomodels from the CD group had a greater nasal length (CD: 18.0 ± 0.3 mm vs. SD: 17.4 ± 0.4 mm; p≤0.03), narrower nasal width (CD: 5.1 ± 0.1 mm vs. SD: 6.1 ± 0.3 mm; p=0.0001) and greater maxillary length (CD: 22.4 ± 0.3 mm vs SD: 21.9 ± 0.2 mm; p≤0.02). Cephalometry indicated that 77-day-old young adult biomodels from the CD group presented longer nasal bone length (SD: 18.2 ± 0.3 mm vs. CD: 18.8 ± 0.3 mm; p≤0.02) and a more pronounced lower incisor inclination (Cephalometric angle MI-Ii/Ii-Id) (SD: 13.8 ± 2.2 degrees vs. CD: 16.1 ± 1 degrees; p=0.06).

Discussion

A growing global obesity epidemic, or 'globesity',[19] is increasingly evident across different world populations. Specifically, obesity in developing countries is one of the most severe public health problems of the 21st century. The WHO reports that overweight children tend to remain obese in adulthood and are more likely to suffer from non-communicable diseases at an early age, all of which are preventable.[20] The impact of globalization and the rapid socio-economic transition has had on nutrition leads us to predict that excess weight will result in easily identifiable morphological effects in bone composition in affected populations within the next few years.

Akiyama et al., provide a high-fat hypercaloric diet to normal adult male Wistar rat. After only 27 days, rats on the experimental group became obese. Every week, the obese rats gain doubles the weight than those on the control group.[21] Different from those results, a two-week cafeteria diet provided by Muntzel et al., to female Wistar rats indicates that modest but nonsignificant increase in body weight and a doubling of adipose tissue.[22] Something similar happened to four-week-old male Wistar rats on Andrich et al. experiment, which shows that a short period of the obesogenic diet shown not to generate weight change, but increased visceral fat.[23] The 38-day-old preadolescent rats of the CD group may have no presented morphological changes due to the short duration of the obesogenic diet; 17 days.

The 77-day-old young adult, male Wistar rats, subjected to an eight week (56 days) diet high in processed snack foods, developed obesity. This finding concurs with a study carried out by Buyukdere et al. in 2019, where rats fed a cafeteria diet for 12 weeks had significantly greater weight gain compared to those fed only a high-fat diet.[24]

Furthermore, this diet led to developing of a significantly larger perigonadal adipose panicle in the 77-day-old young adult biomodels than in the biomodels fed the standard diet. This is also in agreement with the data from the Buyukdere et al. study, in which the diet led to pronounced adiposity in the peri-renal and epididymal fat deposits, thus demonstrating that the cafeteria-type diet triggered persistent hyperphagia and increased energy consumption as a result of the diversity and novelty of the foods presented. In fact, throughout the study period, the group fed the processed food diet showed higher continuous energy and food intake.[24] Animal models offer a valuable opportunity to understand the effects of obesity on a developing system, allowing interventions that are not possible in humans.[25]

There are six case-controlled studies in humans in which obese adolescents present morphological changes in the craniofacial bone component when compared to their normal-weight counterparts. The studies by Ohrn et al., Sadeghianrizi et al., Lee et al., and Giuca et al., show that obese adolescents presented a significant increase in the length of the anterior cranial base, assessed by measuring the distance between the points of the sella and nasion.[6,8-9] The obese, 77-day-old young adult biomodels in this study follow this trend; this suggests that obesity in 77-day-old young adult biomodels increases the cranial base and develops of a higher facial height.

Regarding the nasal structure, the present investigation, employing morphometry showed that an obesogenic diet influenced the 77-day-old young adult biomodels to develop a narrower nasal structure. Moreover, through cephalometry, a greater nasal height was also recorded. This component was not evaluated in the studies on humans we have referenced here. Nevertheless, these changes may have a direct relationship with reduced levels of air intake. Lee et al.,

present obese adults with obstructive sleep apnea,[7] which is known to be associated with craniofacial changes.[27]

Obese 77-day-old young adult biomodels in this present study also had a slightly elongated maxilla. Regarding the mandible, the study by Ferrario et al. demonstrates, through anthropometry, how obese Italian adolescents presented an increase in the length of the mandibular body measured from the soft tissue points of the pogonion to gonion.[5] The young adult biomodels fed the obesogenic diet in this study follow this trend, but changes were evaluated at the bone tissue level. In relation to the posterior mandibular body length, again assessed from the gonion to pogonion points, studies by Sadeghianrizi et al., and Olszewska present an increased posterior mandibular body length in obese Swedish and Polish adolescents, respectively.[6,10] The obese 77-day-old young adult biomodels in this study also demonstrate this characteristic. It would suggest that the obesogenic diet may have influenced the bone structures of these biomodels: they have a longer mandibular body and, in addition, the lower incisors developed a greater inclination, possibly to increase gnawing capabilities as suggested by the paper of Cox et al.[28] Another dimension assessed in the mandible was the total mandibular length, which, in the study by Ohrn et al. and Sadeghianrizi et al., is measured from the condilion to prognation cephalometric points, from the condilion to the gnation point in the study by Olszewska, and from the gonion points to the chin in the study by Lee et al.[6-7,9-10] All these studies mentioned above present an increase in total mandibular length, regardless of how it is measured, which differs from the present study where this trend is not observed.

In contrast to the observations made in the obese adolescents in the aforementioned research studies and the obese 77-day-old young adult biomodels of this study, in a study by Fernandes et al., Wistar rat skulls and jaws were examined after subjection to a diet highly restricted in protein and energy, in a simulation of malnutrition. In the evaluation of these biomodels by morphometry without sex discrimination, a decrease was observed in all the bone dimensions measured.[13]

These results would indicate that there are distinct signaling pathways in both underweight and excess weight scenarios, which influence and determine the development of the bony constitution of the skull and mandible.

Conclusions

These results suggest that administering an obesogenic diet to rats during bone growth and development alters the morphology of the craniofacial complex. More experiments are needed to evaluate the molecular mechanisms involved in bone remodeling process in the presence of an obesogenic diet in the craniofacial complex.

Acknowledgements

This work was supported by the Vice Presidency of Research, Universidad del Valle [grant number 1865, 2019].

Sirsa Aleyda Hidalgo, Ana María Soria and Manuel González, for their help in the laboratory and with the management of the animals. To the Histology Laboratory of the Morphology Department and to the dermestid colony of the Biology Department, Universidad del Valle.

Declaration of Interest

The authors declare that they have no conflict of interest.

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