Intermittent hypoxia causes mandibular growth retardation and macroglossia in growing rats

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Introduction: In this study, we aimed to examine the role of intermittent hypoxia (IH) in dentofacial morphologic changes in growing rats. **Methods:** Seven-week-old male rats were exposed to IH at 20 cycles per hour (nadir of 4% oxygen to peak of 21% oxygen) for 8 hours per day for 6 weeks. Control rats were exposed to normoxia (N). Maxillofacial growth was compared between the 2 groups by linear measurements on cephalometric radiographs. To examine the dental arch morphology, study models and microcomputed tomography images of the jaws were taken. Additionally, tongue size was measured. **Results:** The gonial angle and the ramus of the mandible were smaller in the IH group than in the N group, whereas the body weights were not different between the 2 groups. Morphometric analysis of the dentition showed a significantly wider mandibular dentition and narrower maxillary dentition in the IH than in the N group. The relative width (+4.2 %) and length (tongue apex to vallate papillae, +3.5 %) of the tongue to the mandible were significantly greater in the IH group than in the N group. **Conclusions:** IH induced dentofacial morphologic discrepancies in growing rats. (Am J Orthod Dentofacial Orthop 2017;151:363-71)

Anatomic imbalances between the upper airway soft tissues and the craniofacial dimensions, a lower hyoid bone position, and abnormal upper airway neuromotor tone have been implicated in the development of obstructive sleep apnea (OSA) in children.[1] Children at high risk for sleep-disordered breathing exhibit mandibular retrognathia with a palatal crossbite and narrowed dentoalveolar transverse width in the maxillary dental arch. These dentofacial characteristics are associated with reduced nasopharyngeal and oropharyngeal sagittal dimensions.[2] Additionally, distinct correlations have been shown among maxillary constriction, nasal air resistance, and OSA severity.[3] The effectiveness of various corrective measures for dentofacial morphologic changes in children with OSA has demonstrated that such morphologic changes contribute to the development and progression of OSA.[4] Hyoid-mandibular narrowing and tongue enlargement predict the severity of OSA and the likelihood of its treatability by continuous positive airway pressure in patients.[5] Moreover, hypertrophy of the tongue base and root can induce pharyngeal obstruction, which can be treated with radiofrequency volumetric tissue reduction of the tongue.[6]

Pediatric OSA is a multifactorial disorder with a variety of causes, including obesity and related metabolic diseases, prematurity, nasal abnormalities, and adenotonsillar hypertrophy.[7,8] In adults, the most common cause of OSA is obesity associated with excessive soft tissues in the mouth and throat areas. Obesity is also associated with an increase in the systemic inflammation commonly found in children with OSA.[9] However, OSA in the nonobese population is often reported in children and adults.[10-12] Thus, pediatric OSA is not necessarily restricted to obese children. Although childhood obesity might be related to OSA, it is much less commonly associated with the condition than is

adult obesity. Left untreated, OSA can lead to further serious complications in children, including neuro-cognitive impairment and cardiovascular disease.[13] A parallel study of identical twins from 17 cases of non-obese children with OSA demonstrated differential growth and hormonal balance between OSA and non-OSA siblings despite the same genetic background.[10] However, there is controversy related to the development of pediatric OSA and impaired orofacial growth due to multifactorial disorders.

Repetitive episodes of oxygen desaturation during sleep underlie the pathology of pediatric OSA.[14] The ventilatory responses to these episodes are weaker in children than in adults with OSA.[15] Sustained hypoxia induces sternohyoid muscle maladaptation in growing rats, and brainstem hypoxia elicits selective upper but not lower respiratory muscle depression.[16] It is possible that OSA in young children can cause intermittent hyp-oxia (IH), which may lead to improper jaw development and worsening of respiratory function, although no research addressing these possibilities has been pub-lished. No reports have described the role of IH in the os-teogenesis of the craniofacial skeleton in vivo, so the fundamental question is whether IH can induce cranio-facial bone changes related to the development of the micromandible.

The growth spurt stage of the maxillofacial bones differs from that of the longitudinal bones and neural crest; boys undergo mandibular skeletal growth during adolescence.[17] In rats, the maxillofacial bones undergo striking growth and development from prepubertal to young adult periods.[18,19] In this study, we investigated the effects of IH on the dentofacial bones and tongues of prepubertal rats.[20] The experimental design attemp-ted to isolate IH in pediatric OSA from other factors that might affect skeletal growth and bone metabolism (eg, dietary differences) to clarify the possible effects of the respiratory disturbance on skull growth.[21]

MATERIAL AND METHODS

The experimental procedures were performed ac-cording to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1996) under a protocol approved by the Institutional Animal Care and Use Committee of the University of Tokyo (P12-149). Seven-week-old male Sprague-Dawley rats were divided into 2 groups: the experimental group underwent IH at 20 cycles per hour (nadir, 4% oxygen; peak, 21% oxy-gen; 0% carbon dioxide) (IH group), and the normoxia (N) group was exposed to normoxic conditions (room air). The animals were housed in the same plastic cage

placed next to the cage equipped with the IH apparatus for 8 hours per day during the 12-hour "lights on" period, as previously described.[20,22,23] The noise level for the N group was similar to that of the IH group in the adjacent cages to minimize noise stress-related dif-ferences. Moreover, the rats were pair fed to exclude di-etary effects on growth. After 6 weeks, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital and killed by cervical dislocation.

Cephalometric analysis of the craniofacial skull was performed after the experimental period. Lateral and dorsoventral cephalometric radiographs were taken with a soft x-ray machine (CMB-2; SOFTEX, Tokyo, Japan) to evaluate craniofacial growth. The head posi-tion of each rat was fixed with a pair of ear rods.[19,23] The sagittal and transverse cephalometric radiograph settings were 50 kVp and 15 mA with 5-second impulses. Thirteen lateral and 6 dorsoventral landmarks were iden-tified on the cephalometric radiographs to measure craniofacial growth by an author (Y.K.) (Fig 1, *A* and *B*): the most posterior and superior ends of the skull, the intersection between the frontal bone and floor of the anterior cranial fossa, the most anterior ends of the nasal bone, the most posterior and inferior ends of the occipital condyle, the most superior ends of the mandib-ular condyle, the most posterior ends of the mandibular ramus, the most concave portion of the inferior border of the mandibular corpus, the most inferior and anterior points of the lower border of the mandible, the most inferior and anterior points of the alveolar bone of the maxillary incisor, and the anterior and superior ends of the alveolar bone of the mandibular incisor. Cephalo-metric analysis of the dorsoventral radiograph was per-formed by evaluating the following points to quantify craniofacial growth (Fig 1, *B*): the posterior end of the palate on the midline, the points on the lateral portion of the zygomatic arch that pass the posterior end of the palate on the midline, the most anterior and medial points within the bilateral temporal fossae, the anterior end of the mandible on the concavity of the mandibular left and right incisors, and the posterior end of the mandibular body on the left and right. Each measure-ment was repeated and double-checked 3 times.

Fifteen linear distances between the cephalometric landmarks were measured using digital imaging soft-ware (Photoshop CS6 Extended; Adobe Systems, San Jose, Calif) as previously described.[23] Sagittal distances were measured for the total skull length, cranial vault length, and posterior neurocranium height in the neuro-cranium; nasal bone length and midface length in the viscerocranium; and total mandibular length from the condylar head to the mandibular incisor, total mandib-ular length from the condylar head to menton, ramus