Unilateral nasal obstruction induces morphological changes of the mandibular condyle in growing rats

Kenzo Watakabe, Ikuo Yonemitsu *, Yuhei Ikeda, Tang Huan, Takashi Ono

Department of Orthodontic Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Abstract

Purpose: Chronic nasal obstruction is known to decrease blood oxygen saturation. Mouth breathing in association with chronic nasal obstruction leads to the collapse of the buccinator mechanism and to a clockwise rotation of the mandible, which causes mandibular retrusion. This study aimed to investigate the influences of nasal obstruction on the morphological and histological changes of the mandible in growing rats.

Materials and methods: Thirty 8-day-old male Wistar rats were randomly divided into the control and experimental groups. The experimental group underwent unilateral nasal obstruction by cauterization of the external nostrils at 8days of age. Pulse oxygen saturation (SpO2) was monitored every week. Rats were sacrificed at 9 weeks of age. The mandibular changes were analyzed via lateral cephalometric radiographs and micro-CT scans. We utilized toluidine blue and tartrate-resistant acid phosphatase (TRAP) staining for histological analysis. Immunohistochemical staining of hypoxia induced factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), osteoprotegerin (OPG) receptor activator of nuclear factor kappa-B ligand (RANKL) were also performed to reveal the mechanism of the morphological changes.

Results: SpO2 was significantly lower in the experimental than in the control group. In the experimental group, length, bone mineral density and cartilage layer thickness of mandibular condyle were decreased. The number of TRAP-positive cells in the condyle, HIF-1α-positive cells, VEGF-positive cells and RANKL-positive cells in the condylar cartilage was significantly increased. In contrast, a reduced expression of OPG protein was observed in the experimental group.

Conclusions: Our findings suggest that unilateral nasal obstruction in the growth period affects mandibular morphology.

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1. Introduction

Recently, the prevalence of allergic rhinitis has increased worldwide, occurring in 10% to 30% of adults and up to 45% of children [1]. Allergic rhinitis is a representative symptom of nasal respiratory disorder characterized by three major symptoms: sneezing, nasal mucus secretion, and nasal obstruction (NO). Chronic NO is known to decrease pulse oxygen saturation (SpO₂) [2]. Further, lung weight reduction and hormonal behavioral changes have been reported in rat models of bilateral NO [3,4].

Previous studies have demonstrated how mouth breathing associated with rhinostenosis influences the function of the buccinator. Mouth breathing induces lip dysraphism and triggers the clockwise rotation of the mandible further causing mandibular retrusion and Class II malocclusion in humans [5]. Recent studies have revealed a relationship between NO and malocclusion. Mouth breathing combined with nasal congestion induces a vascular mechanism collapse that leads to adenoid facies resulting from the backward rotation of the mandible [6]. This combination has also been found to cause interference of the lip closure, high palate, and Angle Class II malocclusion in humans [7]. Unilateral NO (UNO) was found to suppress the jaw-opening reflex in a rat model [8]. In addition, it has been reported that NO affects not only muscle reflexes but also the growth of both muscle and bone in the rat model [3,9]. In the craniofacial area, decreased growth of the masseter superficial layer and the anterior belly of the digastric muscle, including the vertical reduction of the nasomaxillary complex, were observed in a rat model of bilateral NO [10,11]. In summary, NO has various effects on the craniofacial region; however, the underlying mechanisms of the mandibular morphological changes associated with NO are still unclear.

Hypoxia induced factor-1 alpha (HIF-1α) expression is associated with the decrease of SpO₂ under hypoxic condition in rabbits [12]. It is activated during hypoxia and involved in osteogenesis and angiogenesis [13]. It was also reported that HIF-1α acts on the vascular endothelial growth factor (VEGF) to promote osteoblastic and osteoclastic differentiation and regulate chondrocyte apoptosis through the glycolysis system in rats [14,15]. In addition, the osteoclast activation mechanism of HIF-1α has previously been reported: hypoxia upregulates the glycolysis pathway and increases the expression of glucose transporters [16]. Subsequently, HIF-1α expression increases in osteoclasts with the release of cathepsin K and hydrogen ions resulting in bone resorption in mice [17].

Osteoprotegerin (OPG) is another protein that is reportedly related to osteoclast activation. And, OPG is downregulated under hypoxia, thus promoting osteoclast activation in rats [18]. OPG acts as the receptor activator of nuclear factor kappa-B ligand (RANKL) decoy receptor in the RANK-RANKL system. Generally, its upregulation suppresses osteoclast activity through binding to RANKL in vitro [19]. An increased RANKL/OPG ratio and a decreased number of OPG-positive cells were detected in the previous study using osteoarthritic TMJ of rats [20].

However, to our knowledge, no study has yet investigated the relation between the chronic hypoxia caused by the NO-related expression of HIF-1α, OPG, RANKL and tartrateresistant acid phosphatase (TRAP) activity and the reduction in bone volume. In this study, we evaluated the influence of UNO on the mandibular morphology of growing rats through the assessment of the HIF-1α expression, OPG expression, RANKL expression and TRAP activity in the condyle.

2. Materials and methods

2.1. Animal preparation

Animal protocols were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University (#0170370A), and the experimental procedures were performed in accordance with the University’s Animal Care Standards.

Thirty 8-day-old male Wistar rats were used in this experiment. The rats were randomly divided into the control and experimental groups (n=15 each). All rats were first anesthetized by hypothermia (10min at –18°C). UNO was induced in the experimental group by cauterization of the external nostril on post-natal day 8. Cauterization was performed by burning the surrounding tissues of the left nostril, using a 400°C-stainless-steel wire that was 1mm in diameter. For the first week, we visually checked every day whether cauterized noses remained closed; we re-cauterized each nose immediately if it re-opened. After 1 week, we observed cauterized noses once per week and verified that they never opened during the experimental period. The control group underwent a sham operation in which the cauterizing instrument was placed 1-2mm above the left nostril. 3% chlorotetracycline (Aureomycin® Ointment; Pola Pharma, Tokyo, Japan) was applied on the left external nostril of rats in both groups postoperatively to prevent infection [21]. Weight and pulse oxygen saturation were monitored every week using a pulse oximeter (MouseOx® STARR Life Sciences Corp., Oakmont, PA). Both the experimental and control group animals were euthanized at 9 weeks using CO₂ gas.

2.2. Radiological analysis of the mandible and tibia

The radiological analysis was performed using the procedures reported previously [22]. Briefly, lateral radiographs were obtained with a soft X-ray system (SOFTEX CMB-2; SOFTEX Co., Ltd., Tokyo, Japan) to evaluate craniofacial morphological changes. The head of each rat (n=15 each) was fixed using a pair of ear rods to maintain a standard head position. The head was maintained in contact with the film to reduce the magnification factor [23]. The various parts of the mandibular bone were measured with the NIH image software (NIS-Elements Analysis D, National Institutes of Health, Bethesda, MD, USA). The measurement points are shown in Table 1 and Fig. 1. Selected linear measurements were then obtained (Table 2). The cephalometric landmarks (Table 1, Fig. 1) were derived from the previous studies in rodents [24,25]. The soft X-ray settings were 50kVp, 15mA, and 5-s impulse [23]. The whole tibia was collected and its length was measured as an indicator of whole body growth. All radiographs were taken three times by the same operator.

2.3. Microcomputed tomography analysis (Micro-CT)

Micro-CT analysis and a desktop X-ray micro-CT system (SMX-100CT; Shimadzu, Kyoto, Japan) were utilized to investigate the
Table 1 - Definitions of landmarks.

<table>
<thead>
<tr>
<th>Landmark</th>
<th>Description</th>
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<tbody>
<tr>
<td>Co</td>
<td>The most posterior and superior point on the mandibular condyle</td>
</tr>
<tr>
<td>Go</td>
<td>The most posterior point on the mandibular ramus</td>
</tr>
<tr>
<td>Mn</td>
<td>The most concave portion of the concavity on the inferior border of the mandibular corpus</td>
</tr>
<tr>
<td>Gn</td>
<td>The most inferior point on the ramus that lies on a perpendicular bisector of the line Go-Mn</td>
</tr>
<tr>
<td>L1</td>
<td>The most anterior and superior point on the alveolar bone of the mandibular incisor</td>
</tr>
<tr>
<td>M1</td>
<td>The junction of the alveolar bone and the mesial surface of the first mandibular molar</td>
</tr>
</tbody>
</table>

Fig. 1 – The landmarks used for the measurements on the soft X-ray images of the rats.

Table 2 - Linear measurements of the mandible.

<table>
<thead>
<tr>
<th>Landmark</th>
<th>Measurement</th>
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<tbody>
<tr>
<td>Go-Mn</td>
<td>Posterior corpus length</td>
</tr>
<tr>
<td>M1-L1</td>
<td>Anterior corpus length</td>
</tr>
<tr>
<td>Co-L1</td>
<td>Total mandibular length</td>
</tr>
<tr>
<td>Co-Gn</td>
<td>Ramus height</td>
</tr>
<tr>
<td>Co-Mn</td>
<td>Ramus length</td>
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changes in the bony microstructure of mandibular condyles and tibias of rats (n=15 each). The region of interest (ROI; 1.0 x 1.0 x 0.2mm) was defined as the region 1.0-mm distant from the articular cartilage of the mandibular condyle, to distinguish the ROI from the cortical bone area. Each mandibular condyle was analyzed using three-dimensional image-analysis software (TRI/3D-BON; Ratoc System Engineering, Tokyo, Japan). A scanning resolution of 20μm, which is recommended for the analysis of bone density, was used to assess the mandibular condyle in the cancellous bone of the mandibular condyle [15] (Fig. 2). The tibia lengths were also measured using the same methods as for condyle measurement.

2.4. Tissue preparation

The temporo-mandibular joints (TMJs) of both sides, along with their surrounding tissues were immersed in 4% buffered paraformaldehyde at 4°C for 24h and decalcified with 4% EDTA for 8 weeks at 4°C. Then, they were embedded in paraffin, and serial sections of 6.0-μm thickness were cut through the sagittal plane [26].

2.5. Histomorphometry with toluidine blue staining

The sagittal sections of the center of the condyle were stained with toluidine blue to measure the width of the mandibular condylar cartilage layers and to observe the chondrocytes. From the articular surface down, the condyle was divided into the fibrous, proliferative, mature, and hypertrophic layers. The condylar cartilage was divided into three areas; the anterior, posterior, and superior regions. The anterior region consisted of the anterior half of the cartilage, the posterior region consisted of the posterior quarter of the cartilage, and the superior region consisted of the area between these two regions [27]. Measurement of the width of the cartilage layer of the anterior, posterior, and superior regions was carried out using image analysis software (NIH-Elements Analysis D, National Institutes of Health, USA) (n=15 each) [28].

2.6. Enzymatic staining of tartrate-resistant acid phosphatase (TRAP) activity

Histochemical analysis of TRAP activity in the mandibular condylar bone was performed to detect osteoclasts using the prepared sections. A positive reaction to TRAP activity is a known marker of mature osteoclasts. TRAP staining was performed using a TRAP/ALP Stain Kit (Wako; Tokyo, Japan) (n=15 each) [29]. Quantitative analysis was performed immediately below the hypertrophic layer in the superior region of the condylar cartilage.

Fig. 2 – The region of interest (ROI) was located in the cancellous bone of the mandibular condyle (white box). Abbreviations: M, mesial; D, distal. Bar indicates 500μm.
2.7. **Anti-HIF-1α antibody and anti-OPG staining**

We evaluated HIF-1α, VEGF, OPG and RANKL protein expression in the mandibular condylar cartilage of each rat (n=15 each) using the prepared sections at the same site as the toluidine staining and TRAP staining. Monoclonal mouse anti-HIF-1α (BD Pharmingen, San Diego, CA, USA), monoclonal mouse anti-VEGF (LVC, MS-350-P0, CA, USA), monoclonal goat anti-OPG (sc-8468, Santa Cruz Biotechnology, CA, USA), and polyclonal rabbit anti-RANKL (sc-7628, Santa Cruz Biotechnology, CA, USA) antibodies were used as primary antibodies, at a dilution of 1:100, 1:100, 1:50, and 1:50, respectively. Simple Stain MAX-PO (NICHIREI BIOSCIENCES, Tokyo, Japan) was used as secondary antibodies. In each section, the number of HIF-1α, VEGF, OPG and RANKL-positive cells were counted using a fixed measuring frame (450 μm × 900 μm) at least three times. The measuring frame covered the total thickness of the proliferative and hypertrophic layers in the superior region of the mandibular condyles as previously reported [29,30]. In addition, the RANKL / OPG ratio was calculated in order to reveal changes in osteoclastogenesis, as previously reported [31].

2.8. **Statistical analysis**

After testing for normality and equal variances, the Student’s t-test was used to compare mean values among groups. All statistical tests were performed using SPSS ver. 2.0 (SPSS Japan Inc., Tokyo, Japan). A P-value < 0.05 was considered significant.

3. **Results**

3.1. **Systemic changes in rats**

The body weight, tibial length, and cancellous bone volume density in the tibia of rats were measured as an indicator index of whole body growth. There was no significant difference between the control and experimental groups (Fig. 3A). However, the value of SpO2 in the experimental group was

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**Fig. 3** – (A) (i) Changes in body weight of rats during the experimental period. Values are depicted as the mean ± standard deviation (S.D.). There were no significant differences between the two groups at any stage (n=15 each). (ii) The length of the tibias of 9-week-old rats. Values are mean ± S.D. There were no significant differences between the two groups (n = 15 each). (iii) Comparisons of bone morphology between the control and experimental group by micro-CT analysis. The cancellous bone of the tibia was compared between the control and experimental groups (n=15 each). (B) Changes in pulse oxygen saturation (SpO2) of rats during the experimental period (n=15 each). Values are depicted as the mean ± S.D. There were significant differences between the two groups from 2 to 5, and 7 to 9 weeks of age. *: p < 0.05. Abbreviation: BV/TV, bone volume/tissue volume.
significantly decreased from 2 to 5, and 7 to 9 weeks of age compared to that in the control group (Fig. 3B).

3.2. Cephalometric measurements

In the experimental group, Go-Mn and Co-Mn were significantly shorter than in the controls. These changes indicate that the length of the mandibular ramus was shorter in the experimental group compared with the control group (Fig. 4). However, the length of the M1-L1, Co-L1, and Co-Gn showed no significant difference between the control and experimental groups.

3.3. Three-dimensional micro-CT analysis

The experimental group showed a lower cancellous bone volume density compared with the control group. The micro-CT analysis demonstrated significantly lower BMD and BV/TV of the mandibular condyle in the experimental group compared to the control group (Fig. 5).

3.4. Histomorphometry with toluidine blue staining

Chondroblastic layers consisted of four layers from the superior of the condyle: fibrous, proliferative, mature, and hypertrophic layers (Fig. 6A). The layers in the superior region of the experimental group were thinner than those in the control group. The cartilage in the anterior, posterior, and superior region in the experimental group was significantly thinner than at the corresponding regions in the control group (Fig. 6B). Metachromasia staining was observed in the transitional and hypertrophic layers of the condylar cartilage of both groups. From the mature to the hypertrophic layers, the width of the cartilage in the experimental group changed thinner than in the control group.

3.5. Quantitative analyses of osteoclastic cell numbers

The TRAP-positive cells immediately below the hypertrophic layer of the condyles were observed in both the experimental and control groups (Fig. 7A). The number of TRAP-positive cells in the superior region of the condyle was significantly higher in the experimental group (Fig. 7B).

3.6. Expression of HIF-1α, VEGF, OPG and RANKL protein in the articular cartilage of the rat mandibular condyle

Immunohistochemical analyses showed that the HIF-1α (Fig. 8A) and VEGF (Fig. 8C) protein were expressed specifically in the mature hypertrophic cell layer of the mandibular condylar cartilage of the experimental group. The ratio of HIF-1α (Fig. 8B) and VEGF (Fig. 8D) positive cells were significantly higher in the experimental group than in the control group.

Immunohistochemical analyses showed that OPG protein expression was increased in the mandibular condyle of the control group. In contrast, OPG was barely detected in the experimental group (Fig. 9A). The ratio of OPG-positive cells was significantly higher in the control group compared to the experimental group (Fig. 9B). However, there was no significant difference in RANKL protein expression between control and experimental groups (Fig. 9C). The RANKL/OPG ratio was significantly higher in the experimental group, compared with the control group (Fig. 9D).

4. Discussion

It has been reported that bilateral nasal obstruction in rats greatly affects the growth and development of the whole body [3,9]. However, in our study, when the BMD and length of the tibia were measured as whole body indices, no significant differences were found between the control and experimental groups.
differences were observed, suggesting that UNO has little effect on whole body growth. As we used a unilateral nasal obstruction model rather than a bilateral nasal obstruction model, we had to determine whether unilateral change had occurred. As a result, no significant difference was observed between left and right sides.

Even though there was little effect on the other parts of the body, the UNO animals showed a shortened mandibular ramus, decreased BMD of the mandibular condylar cancellous bone, and thinner condylar cartilage. In the UNO group, the value of SpO₂ was significantly decreased from 2 to 5 and 7 to 9 weeks of age, compared to the control group. The value of SpO₂ stabilized at about 93% after 7 weeks of age. These results are almost in agreement with those of previous studies involving rats [32,33]. Nasal obstruction is related to an initial decrease in lung growth [3], and rat lung growth is reported to occur at 3 weeks of age [34,35], while capillary growth, which plays an important role in optimizing gas exchange, occurs at 2-5 weeks of age [34,36]. In our experiments, we assumed that the experimental period influenced lung and capillary growth. The significant decrease in SpO₂ from 6 weeks of age in the experimental group was likely due to a reduction in ventilation after the completion of the growth of lungs and capillaries, as is seen in humans with nasal obstruction [37].

In the radiological analysis of the mandible, the posterior corpus length and ramus length were significantly shorter in the experimental group. However, the anterior corpus length, total mandibular length, and ramus height were not significantly decreased in the experimental group. According to the previous study, nasal obstruction is associated with upward and backward growth of the condyle, and a decreased vertical component of growth in monkeys [38]. Additionally, the directional growth of the rat mandible is affected by orofacial functional changes [9].

Our findings and those of other groups have revealed various possible mechanisms for the morphological changes of the mandible caused by NO. It was reported that the mandible rotates clockwise due to the nasal obstruction and assumes a mandibular posture similar to open bite. This causes decreased growth of the mandibular ramus, similar to the results of the current study. Furthermore, it was reported that in the case of such a mandibular posture, the activity of the jaw-closing muscle of the mandible reduces and the weight of the masseter muscle decreases [38]. In line with this, we observed a decreased length of the mandibular ramus in our experimental group, which implies a lower masseter muscle mass and less mandibular ramus growth.

According to another previous study, body growth was delayed in a short-term unilateral nasal obstruction model after

**Fig. 6** - (A) Histological staining with toluidine blue of 6-μm sagittal sections of 9-week-old rat condyle: (i) control and (ii) experimental groups (n = 15 each). Metachromatic staining was observed in the transitional and hypertrophic layers of the condylar cartilage of both groups. Bar indicates 100 μm. Abbreviations: F, fibrous layer; P, proliferative layer; M, mature layer; H, hypertrophic layer. (B) The width of the chondroblastic layers of the superior region of 9-week-old rat condyles was measured. Values are depicted as the mean ± S.D. *: p < 0.05.
birth; and low levels of glucocorticoids caused the decreased growth of the rat craniofacial area. However, at 90-days-old, there were no significant differences in body weight [9]. Considering the results of this previous study, it is possible that the level of growth hormone was also decreased in our model, which may have resulted in reduced growth of the mandible.

It is widely known that the mandibular condylar growth is caused by endochondral ossification; however, there have been no studies focused on the relationship between expression of HIF-1α and endochondral ossification in a nasal obstruction model. It has been reported that HIF-1α is involved in the apoptosis of chondrocytes [42] and is related to a decrease of cartilage thickness in the rat mandibular condyle [29], which is concordant with our findings.

It is possible that the whole body decrease in SpO₂ arising from the nasal obstruction caused a localized reduction of SpO₂ at the TMJ, thereby affecting the mandibular condylar cartilage. A previous study reported that SpO₂ in the synovial fluid of rheumatoid arthritis patients is lower than that of healthy subjects [43]. Similarly, the reduction of SpO₂ in the mandibular condylar cartilage might have also occurred in nasal obstruction model [38-41]. Although HIF-1α promotes cell survival under normal circumstances [44], its expression under hypoxic conditions can activate pathways that lead to synovitis, angiogenesis, cartilage degradation, and bone erosion [45-48]. It was reported that the expression of HIF-1α increases in the mandibular condylar cartilage of rats under hypoxia [49]. Our results also suggested that the increased expression of HIF-1α in the mandibular condylar cartilage, due to systemic hypoxia with nasal obstruction, may have caused cartilage degradation and reduction of bone density on the mandibular condyle.

In mandibular bone morphometry, a decrease in mandibular ramus length and in mandibular bone density were observed, but no change was observed in the tibia. This is similar to that in the intermittent hypoxia (IH) model involving rats [50]. Breathing interacts with chewing and swallowing, etc., thereby allowing proper growth and development of the craniofacial area. Chronic nasal respiratory disturbance is often observed in children’s nonspecific respiratory deformities such as adenoid hypertrophy [9]. The difference between the results in the mandibular bone and the tibia may be due to the fact that the nasal obstruction causes physiological and functional changes in the mandibular anterior muscles, resulting in a change of muscle function in the craniofacial area. This thus leads to a large influence on the mandibular bone. We are currently studying the degeneration of the

Fig. 7 – (A) Tartrate-resistant acid phosphatase (TRAP) staining in the superior region of 9-week-old rat mandibular condyle. (i) Control and (ii) experimental groups. TRAP-positive cells were seen in osteoclasts beneath the cartilage layer. Bar indicates 100 μm. (B) The number of TRAP-positive cells found in 9-week old rat condyles (n=15 each). Quantitative analysis was carried out immediately below the hypertrophic layer in the superior region of the condylar cartilage. Values are depicted as the mean ± S.D. *: p < 0.05.
Fig. 8 – (A) Representative immunohistochemical staining with anti-HIF-1α antibody in both the (i) control and (ii) experimental groups. Arrow heads indicate HIF-1α-positive cells. Bar indicates 100 μm. (B) HIF-1α-positive cells were counted in each group (n=15 each) using a fixed measuring frame (450 μm × 900 μm). The percentage of HIF-1α positive cells was calculated as the number of positive cells to the total number of counted cells. Values are depicted as the mean ± S.D. *: p < 0.05. (C) Expression of VEGF in the condylar cartilage of rat temporo-mandibular joints. Representative immunohistochemical staining with anti-VEGF antibody in both the (i) control and (ii) experimental groups. Bar indicates 100 μm. (D) The percentage of VEGF-positive cells was calculated as the number of positive cells per total number of cells (n=15 each). Values are depicted as the mean ± S.D. *: p < 0.05.
Fig. 9 - (A) Expression of OPG in the condylar cartilage of rat temporo-mandibular junctions. Representative immunohistochemical staining with anti-OPG antibody in both the (i) control and (ii) experimental groups. Bar indicates 100 μm. (B) The percentage of OPG-positive cells was calculated as the number of positive cells per total number of cells (n=15 each). Values are depicted as the mean ± S.D. *: p < 0.05. (C) Expression of RANKL in the condylar cartilage of rat temporo-mandibular joints (n=15 each). Representative immunohistochemical staining with anti-RANKL antibody in both the (i) control and (ii) experimental groups. Bar indicates 100 μm. (D) The ratio of RANKL/OPG was calculated (n=15 each). Values are depicted as the mean ± S.D. *: p < 0.05.
masticatory muscle in a specific 9-week-old nasal obstruction model.

Several experimental hypoxic stress models have demonstrated the effects of IH on rats within the hypoxic chambers [51]. In particular, IH has been shown to lead to a decreased mandibular ramus height and increased mandibular bone density [24]. In the IH model, oxygen saturation is lower than that in normal subjects, similar to the reduced SpO2 due to UNO in the current study. This might be the reason for the similarity in findings. In both models, no significant difference was observed in the tibial length; significant differences were only observed in the craniofacial area bones. However, with regards to the BMD, the results of our study differed: the BMD of the mandibular condylar cancellous bone was decreased in the UNO group. This could be the consequence of differences in the breeding age of rats and the oxygen saturation levels. In our model, the rat’s breeding age was 8 days old to 9 weeks old and the oxygen saturation in the UNO was chronically about 94%, whereas in the IH model, the breeding age was from 7 to 10 weeks old and the oxygen saturation intermittently ranged from 4% or 5% to 21% [50,52].

There are various opinions regarding the effects of hypoxia on BMD. A previous study claimed that chronic IH does not influence BMD [53]. In contrast with the previous studies, we focused on the period from early childhood until the end of the growth. Therefore, the decrease of BMD observed in this study might be related to the difference in the age of rats compared to previous experimental models. Moreover, we found that the experimental group had a chronic decrease in SpO2 from 8 days to 9 weeks of age. Because decrease in SpO2 was chronic, and not intermittent or long-term, our results were different from those of the IH model. Based on the above, we believe that the changes in bone mineral density were due to multiple factors, including changes in masticatory muscle activity, age, and hypoxic exposure time.

HIF-1α is one of the important markers for evaluating BMD and chondrocyte layer thickness in the NO model because it relates to the hypoxic stress condition, osteoclast differentiation, and chondrocyte apoptosis [42]. In the UNO group, the number of HIF-1α-positive cells increased and the chondrocyte thickness decreased significantly, which is similar to the previous findings regarding HIF-1α expression [29,54]. Similarly, a correlation exists between the expression of TRAP-positive cells and HIF-1α-positive cells [55]. We observed a similar increase in the number of both HIF-1α-positive and TRAP-positive cells in the UNO group. The TRAP positive-cells of secondary spongiosa, corresponding to the ROI of micro-CT in the UNO group, were also increased; moreover, osteocytes and bone marrow were disarranged in the secondary spongiosa in the UNO group. These were consistent with the results of micro-CT. As for OPG and RANKL, we observed the activation of osteoclasts and reduction of OPG, the increased RANKL/OPG ratio was similar to previous reports [19,56,57]. Therefore, we suggest that the expression of HIF-1α decreases the expression of OPG, leads to an increased RANKL/OPG ratio, resulting in an increase of the number of TRAP-positive cells and a reduction of bone density. Moreover, we found a decrease of the chondrocyte layer thickness in the experimental group, confirming a previous finding that HIF-1α expression promotes chondrocyte apoptosis [58]. In addition, the aforementioned mechanism is also conceivable due to the fact that HIF-1α and OPG are expressed in the mature and hypertrophic layers, and that the number of osteoclasts immediately under the hypertrophic layer is increased. Furthermore, the toluidine blue staining in the experimental group showed enlarged and roughened chondrocytes. This appearance was more frequently observed in the hypertrophic chondrocyte layer. This is similar to the observations of chondrocytes during apoptosis in a previous study [59].

5. Conclusions

This is the first study to report the histological effects of nasal obstruction on the mandible. Our results indicate that nasal obstruction during the growth period causes morphological and histological changes in the mandible.

Conflicts of interest

None to declare.

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Ethical approval

The experimental procedures described here were approved by the Institutional Animal Care and Use Committee (#0170370A) and performed in accordance with the Animal Care Standards of Tokyo Medical and Dental University.

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