Original Article

Effects of unilateral nasal obstruction on the characteristics of jaw-closing muscles in growing rats

Huan Tang[a]; Ikuo Yonemitsu[b]; Yuhei Ikeda[c]; Kenzo Watakabe[a]; Shunichi Shibata[d]; Jun Hosomichi[e]; Takashi Ono[f]

ABSTRACT

Objectives: Mouth breathing caused by nasal obstruction (owing to abnormal pressure of masticatory muscles) affects craniofacial growth and development. The influence of unilateral nasal obstruction on jaw-closing muscles was investigated in rats to reveal one of the etiologic mechanisms.

Materials and Methods: Forty 8-day-old male Wistar rats were used in this study. Experimental rats were subjected to left-sided nasal obstruction by burning the external nostril tissue at the age of 8 days. Pulse oxygen saturation was recorded each week. Morphologic changes were evaluated by staining with hematoxylin and eosin (to assess the cross-sectional area) and by adenosine triphosphatase activity staining (to assess the myosin heavy chain isoform composition). Immunohistochemical and reverse transcription quantitative real-time polymerase chain reaction analyses of tumor necrosis factor-α and glucose transporter 4 were carried out at 5 and 9 weeks of age.

Results: The cross-sectional area of the jaw-closing muscles was lower in the experimental group at 9 weeks of age. The percentage of myosin heavy chain-2a in masseter muscles was increased in the experimental group compared with the control group. An increase in the tumor necrosis factor-α messenger RNA and protein levels and a decrease in the glucose transporter 4 messenger RNA and protein levels at 5 and 9 weeks of age in the jaw-closing muscles in the experimental group were noted.

Conclusions: Unilateral nasal obstruction could affect the morphology and contractile characteristics of jaw-closing muscles during growth in rats. (*Angle Orthod.* 2019;89:102–110.)

KEY WORDS: Unilateral nasal obstruction; Jaw-closing muscle; Tumor necrosis factor-α (TNF-α); Glucose transporter 4 (GLUT4); Myosin heavy chain (MHC)

INTRODUCTION

The relationship between mouth breathing and craniofacial morphology has been studied for decades.[1–3] Chronic nasal obstruction followed by mouth breathing is a nonspecific condition that can occur in rhinosinusitis, allergic rhinitis, and adenoid hypertrophy. It results in a backward and downward rotation of the mandible, an increase in the mandibular plane

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angle, augmentation of the anterior lower vertical facial height, and reduction of the posterior facial height.[1,3,4] It has been shown that nasal obstruction decreases the skull base along the longitudinal axis and inhibits vertical development in the nasomaxillary complex in rats.[5] Clinical studies have shown that nasal obstruction followed by oral respiration decreases masseter electromyographic (EMG) activity and increases suprahyoid EMG activity.[6] Additionally, a reduction of masseter EMG activity followed by injection of botulinum toxin reduces the size of muscle fibers and induces conversion of fiber types.[7]

Myosin heavy chain (MHC) composition is associated with the contractile properties of muscles. Adult skeletal muscles contain three types of MHC: 1 (slow), 2a (fast), and 2b (fast).[8] With regard to fast fibers, MHC-2b exhibits faster shortening velocity, generates a greater maximum specific force, and has lower fatigue resistance than MHC-2a.[9] The MHC isoforms include fast- and slow-twitch isoforms. Hence, adaptations in muscle force can occur in response to altered requirements due to changes in environmental conditions, such as hormone levels, occlusal force, and hypergravity.[10]

Tumor necrosis factor-α (TNF-α) is synthesized by skeletal muscle myocytes and was shown to play a role as an endogenous mediator to influence muscle growth and adaptation via paracrine and autocrine effects.[11] TNF-α expression in skeletal muscles influences muscle metabolism via three general actions: promoting catabolism (protein loss and insulin resistance), inhibiting contraction, and modulating myogenesis.[12,13] The myogenetic potency of TNF-α is evident from animal studies showing that TNF-α induced a reduction in contractile function.[14] Glucose transporter 4 (GLUT4) in skeletal muscles was shown to be an important glucose-transporter isoform that plays a major part in insulin- and contraction-stimulated glucose transport.[15] A negative impact on muscle regeneration occurred if the GLUT4 pathway was impaired.[16] Extrapolating these findings, TNF-α and GLUT4 could be markers of the muscle contractile properties and adaptation of muscles.

In the current study, histologic and histochemical approaches were used to explore the influence of nasal obstruction on the morphologic changes and characteristics of jaw-closing muscles in growing rats. Obvious advantages of a histochemical approach are high sensitivity and direct evidence of functional changes in protein levels at qualitative and quantitative levels.

MATERIALS AND METHODS

Animal Model

The study protocol was approved by the Animal Welfare Committee of Tokyo Medical and Dental University (No. 0170370A, Tokyo, Japan).

Forty male Wistar rat pups were divided randomly into experimental and control groups (n = 20 each). At 8 days of age, the rat pups were placed first in a chamber at –18°C for 10 minutes to anesthetize them by hypothermia. After confirming that the temperature of the external nostril was decreased sufficiently, experimental group rats were subjected to left-sided nasal obstruction by burning the tissue surrounding the external nostril using a surgical instrument (Hakko Red; Hakko Corporation, Osaka, Japan).This approach did not cause mechanical/chemical damage to the olfactory mucosa and is the most common and simplest procedure for neonatal animals.[4] A sham operation was undertaken by placing a cauterizing device approximately 1–2 mm above the external nostril on the left side in the control group.[17] After cauterization, 3% chlortetracycline (Aureomycin Ointment; Pola Pharma, Tokyo, Japan) was used to prevent infection. The body weight of the rats was measured each week throughout the experimental period. A mouse pulse oximeter (MouseOX; STARR Life Sciences, Oakmont, Pa) was used while administering 4% isoflurane (inhalation anesthetic) (SFMBX1; DS Pharma Biomedical, Osaka, Japan) to measure pulse oxygen saturation (SpO_2) each week.[18]

Muscle Sampling and Tissue Preparation

Rats were euthanized according to a standard CO_2/O_2 protocol at 5 and 9 weeks of age (n = 10 per group per time point). After euthanasia, the bilateral superficial masseter, deep masseter, medial temporalis, and tibialis anterior (reference) muscles were dissected from their attachment sites. After collection, the muscles were trimmed and samples obtained from the muscle belly. The muscles were frozen directly in optimal cutting temperature embedding compound (Tissue-Tek II; Miles Laboratories, Naperville, Ill). For each muscle type, transverse sections were cut using a cryomicrotome (CM3500; Leica Microsystems, Nussloch, Germany). Then, sections were subjected to staining with hematoxylin and eosin (to assess the mean cross-sectional area [CSA]) and to adenosine triphosphatase (ATPase) activity staining (to assess MHC composition), in addition to immunohistochemical (IHC) analyses of TNF-α and GLUT4. Image J (National Institutes of Health, Bethesda, Md) was used to measure the CSA of muscles.

ATPase Activity Staining

ATPase activity staining was performed according to a standard protocol that was described previously.[19] Slides were first preincubated (0.1 M barbital acetate and 0.1 M HCl, adjusted to pH 4.6) for 5 minutes, and

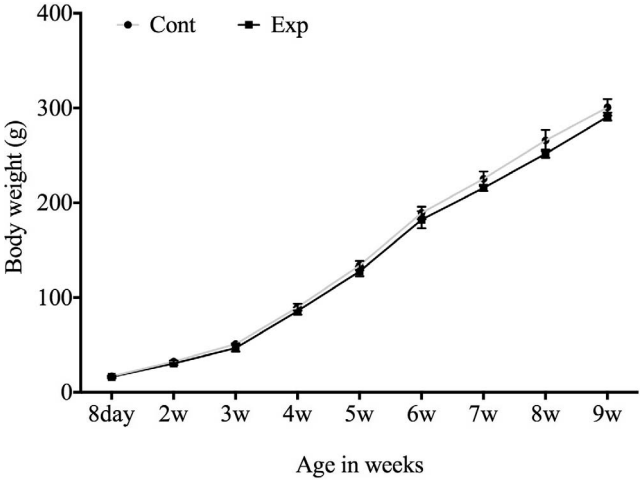

Figure 1. Changes in the body weight of rats during the experimental period. Abbreviations: Cont, control group; Exp, experimental group.

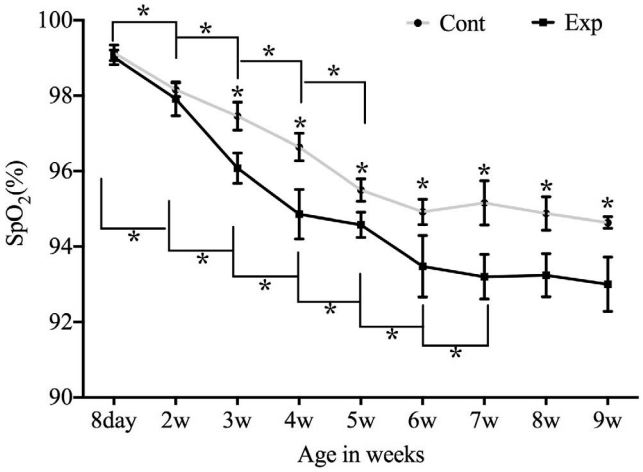

Figure 2. Pulse oxygen saturation (SpO_2) * $P < .05$. Abbreviations: Cont, control group; Exp, experimental group.

then rinsed with a substrate solution (0.18 M calcium chloride and 0.1 M sodium barbital, adjusted to pH 9.4). Slides were incubated in an adenosine triphosphate solution (0.18 M calcium chloride, 0.1 M sodium barbital and 60 mg adenosine triphosphate powder) at pH 9.4 for 45 minutes. Then, slides were washed three times with 1% calcium chloride solution for 10 minutes each, incubated with 2% cobalt chloride for 3 minutes, washed five times with 5 mM sodium barbital solution, and rinsed with water for 2 minutes. Finally, slides were incubated with 1% ammonium sulfide for 1 minute and rinsed with water five times. Sections were observed under an optical microscope (NIS-Element; Nikon, Tokyo, Japan) and the fiber types identified to calculate the percentage of each type.

IHC Analyses of TNF-α and GLUT4

After rinsing in phosphate-buffered saline (PBS), sections were treated with 0.3% hydrogen peroxide for 30 minutes. Then, sections were blocked with 3% bovine serum albumin for 30 minutes. After rinsing with PBS, sections were incubated with goat anti-rabbit GLUT-4 primary antibody (Abcam, Cambridge, United Kingdom) and goat anti-rabbit TNF-α primary antibody (Abcam) overnight at 4°C. Slides were incubated with biotinylated universal secondary antibody (Vectastain Universal Quick Kit, Vector Laboratories, Burlingame, Calif) for 30 minutes at room temperature after washing thrice with PBS. Immunoreactive sites were visualized with 0.03% 3,3-diaminobenzidine (ImmPACT DAB, SK4105; Vector Laboratories). Sections were observed under an optical microscope, and five non-overlapping high-magnification fields were selected.

Reverse Transcription Quantitative Real-Time Polymerase Chain Reaction

The total RNA of samples from the superficial masseter, deep masseter, medial temporalis, and tibialis anterior muscles were isolated based on manufacturer instructions using a PureLink FFPE Total RNA Isolation Kit (Invitrogen, Carlsbad, Calif).[20,21] Isolated total RNA was reverse-transcribed into complementary DNA with random primers using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, Calif). For each sample, a TaqMan polymerase chain reaction (PCR) assay (TaKaRa Biotechnology, Shiga, Japan) of each target gene (TNF-α and GLUT4) and control gene (actin) was undertaken in triplicate using a 7500 Real-Time PCR system (Applied Biosystems). PCR was carried out with a fluorescently labeled TaqMan Probe (TaKaRa Biotechnology) and gene-specific primers.

Statistical Analyses

Two-way analysis of variance was used for intergroup and intragroup analyses of SpO_2. The Student's *t*-test was conducted for other comparisons between the two groups using SPSS version 20.0 (IBM, Armonk, NY). $P < .05$ was considered significant.

RESULTS

Body Weight and SpO_2

The body weight of all rats increased in a normal manner throughout the experimental period. There were no significant differences at any stage between the rats in each group (Figure 1). In the control group, SpO_2 decreased from the age of 8 days to 5 weeks, reached approximately 95%, then remained stable until

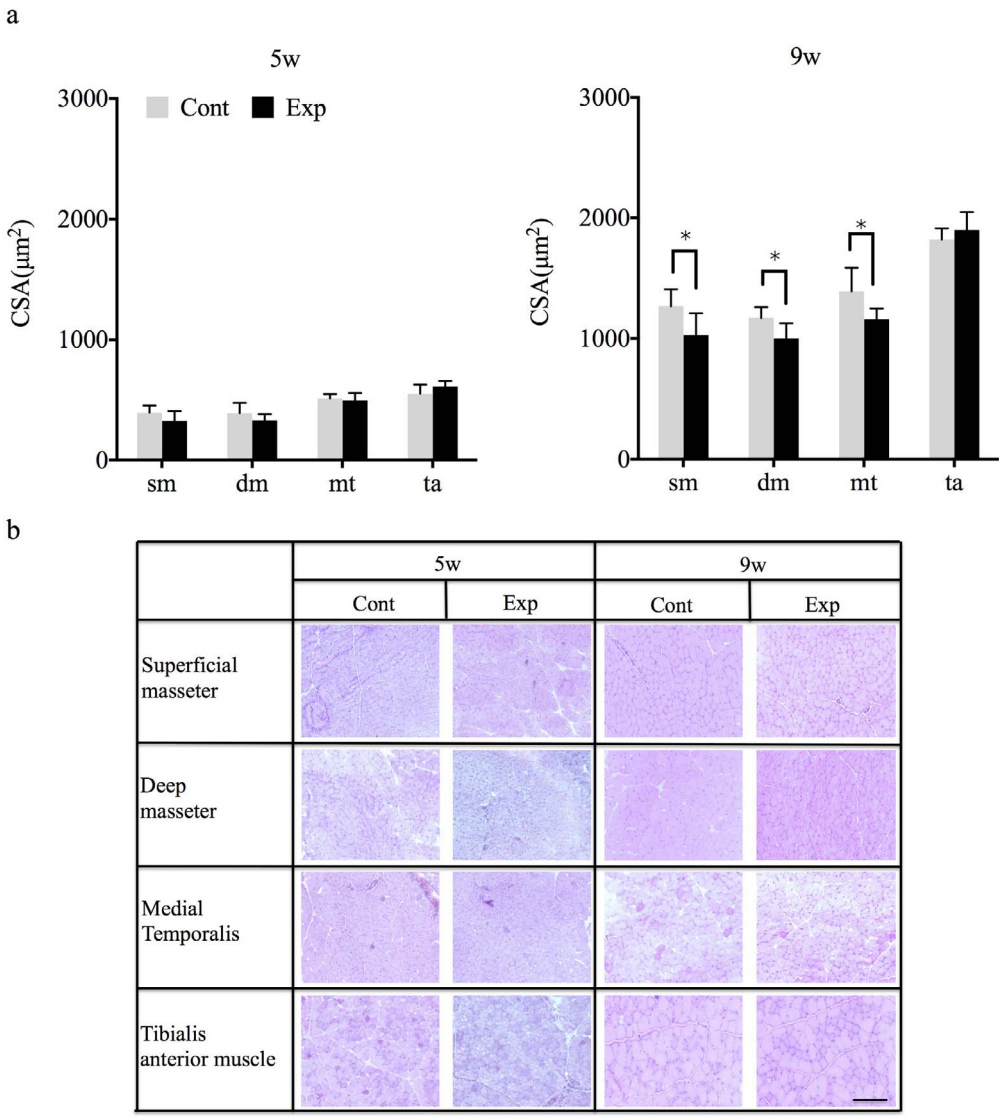

Figure 3. (a) Mean cross-sectional area of muscle fiber at 5 and 9 weeks old. (b) Hematoxylin and eosin staining. Scale bar, 200 μm. * $P < .05$. Abbreviations: Cont, control group; Exp, experimental group; sm, superficial masseter; dm, deep masseter; mt, medial temporalis; and ta, tibialis anterior.

9 weeks of age. In contrast, SpO_2 decreased until the rats were 7 weeks old, reached approximately 93%, then remained stable until 9 weeks of age in the experimental group. From 3 weeks of age, the SpO_2 in the experimental group was significantly lower than that in the control group (Figure 2).

CSA and Expression of MHC Isoforms

In the superficial masseter, deep masseter, and medial temporalis muscles, the CSA was reduced at 9 weeks of age in the experimental group compared with the control group (Figure 3).

The ATPase activity staining procedure stained type-1 myofibers dark brown, type-2a myofibers white, and type-2b myofibers medium brown. Only MHC-2a and MHC-2b were observed at the marginal portion of superficial and deep masseter muscles (slow-type MHC-1 was not detected). At 5 and 9 weeks of age, the percentage of type-2a fibers in the experimental group was increased in the superficial and deep masseter muscles compared with that in the control group (Figure 4).

IHC of TNF-α and GLUT4 and Reverse Transcription Quantitative Real-Time PCR

In the experimental group, there was enhanced TNF-α immunoreactivity and lower GLUT4 immunoreactivity in sections of the superficial masseter, deep masseter, and medial temporalis muscles at 5 and 9 weeks of

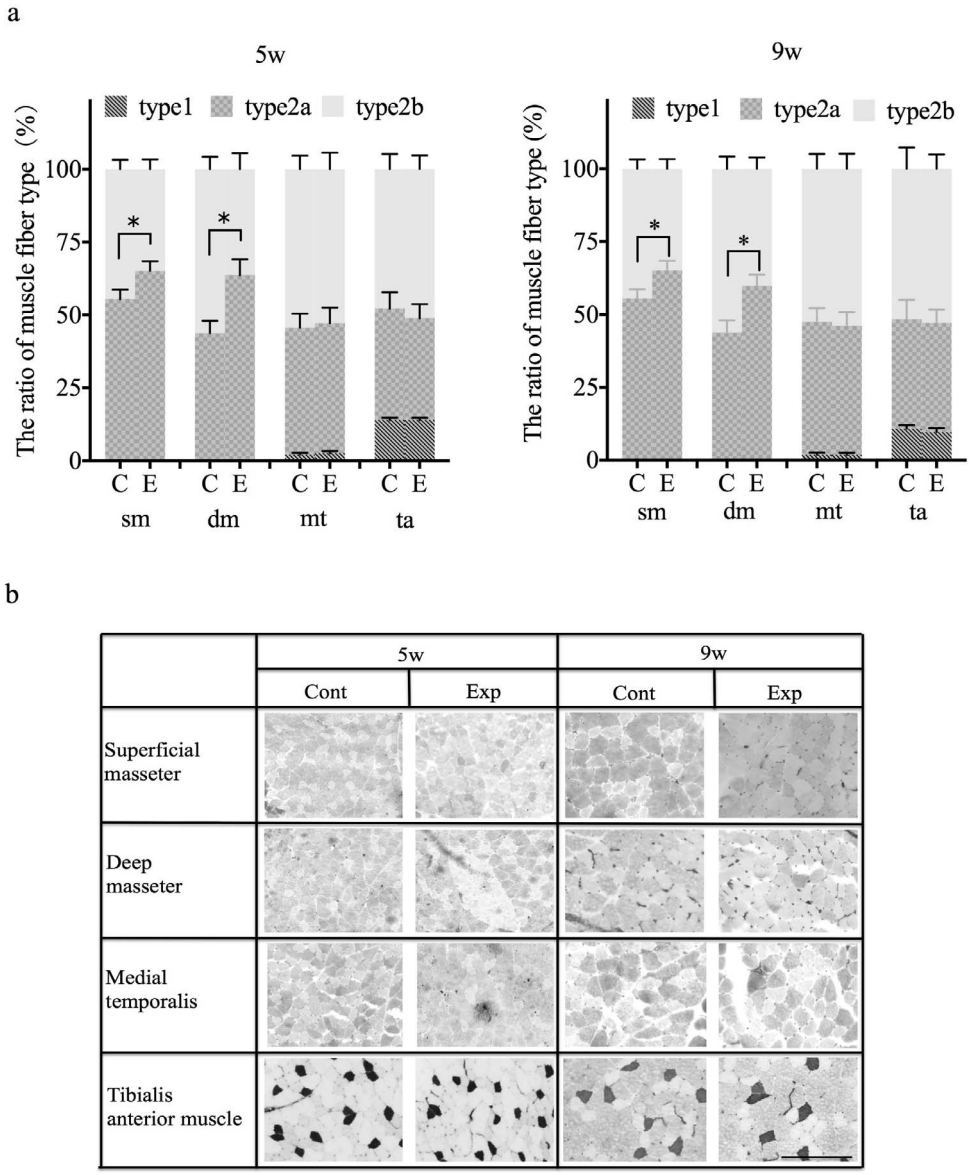

Figure 4. (a) Composition of fiber types in jaw-closing muscles. (b) Adenosine triphosphatase staining. Scale bar, 200 μm. Abbreviations: Cont, control group; Exp, experimental group; sm, superficial masseter; dm, deep masseter; mt, medial temporalis; and ta, tibialis anterior.

age compared with those in the control group (Figure 5).

In the experimental group, the messenger RNA (mRNA) levels of GLUT4 decreased by 17%, 21%, and 10% in the superficial masseter, deep masseter and medial temporalis muscles, respectively, at 5 weeks of age. These levels decreased by 31%, 35%, and 27% in the superficial masseter, deep masseter, and medial temporalis muscles, respectively, at 9 weeks of age compared with those in the control group. At 5 weeks of age, the TNF-α mRNA levels in the experimental group increased by 67%, 63%, and 52% in the superficial masseter, deep masseter, and medial temporalis muscles, respectively, compared with those in the control group. At 9 weeks of age, the increases were 71%, 63%, and 45%, respectively (Figure 6).

DISCUSSION

In humans, nasal obstruction followed by mouth breathing can lead to hypoxia.[22] SpO_2 is a standard measurement used in clinical practice because it is a reliable method for predicting arterial oxygen saturation (SaO_2).[23] In the present study, the SpO_2 values were investigated in two groups of rats. SpO_2 decreased significantly from 3 weeks of age in the experimental group. Unilateral nasal obstruction may have induced a low level of oxygen due to a reduction in airflow.

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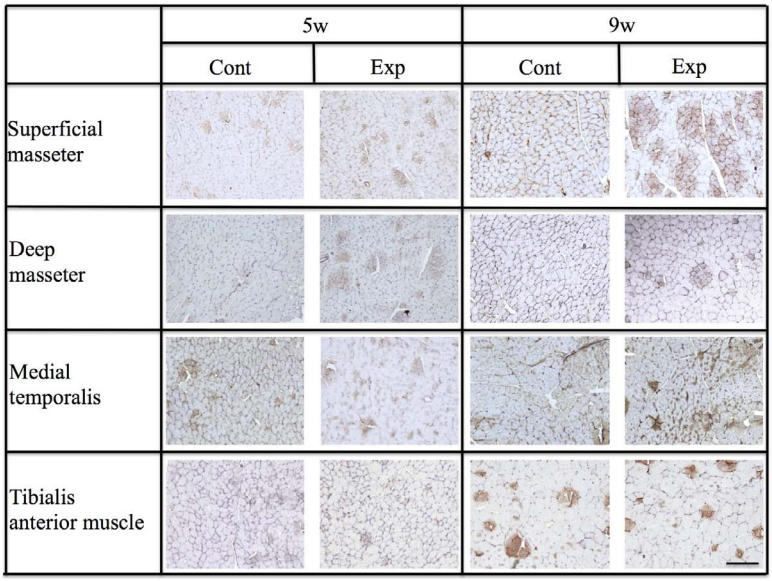

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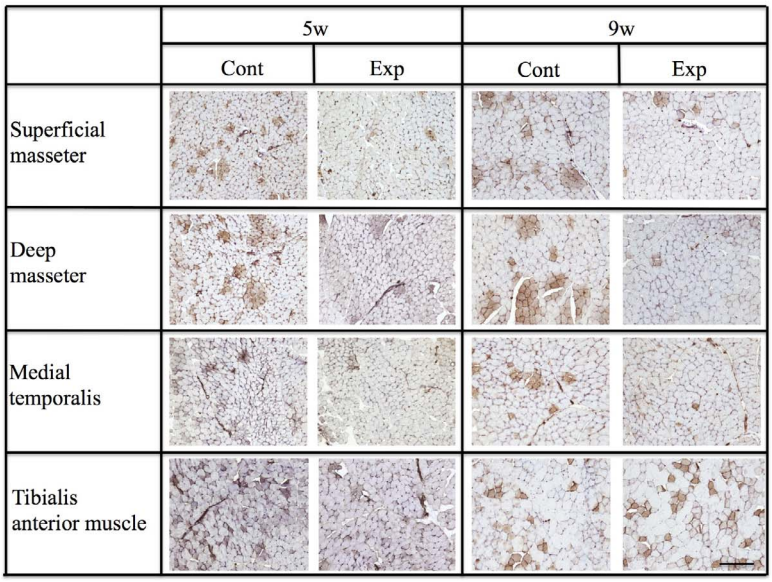

Figure 5. Expression of tumor necrosis factor-α (a) and glucose transporter 4 (b) in jaw-closing muscles. Scale bar, 200 μm. Abbreviations: Cont, control group; Exp, experimental group.

Harvold and colleagues[24] reported that infant monkeys adapted to nasal obstruction by enlarging the oral airway or maintaining a low-positioned mandible with or without protruding the tongue. In contrast, creating a passage of air through the oral cavity in rats is difficult because of the configuration of the epiglottis and soft palate.[25] Thus, it is likely that rats could not adapt to bilateral nasal obstruction and learn mouth breathing. Studies have shown that nasal obstruction in rats increased mandibular development by allowing respiratory compensation in breathing.[25,26] Additionally, it was reported that unilateral nasal obstruction in rats at 8 days of age enhanced the jaw-opening reflex and contractile properties of the tongue-protruding muscles.[17] Therefore, it was speculated that rats adapted to nasal obstruction by reducing nasal airflow as well as increasing the gape and activity of the tongue-protruding muscles.

Studies have shown that nasal obstruction can reduce the electromyographic activity of the masseter and anterior temporalis muscles.[27] To meet a wide range of functional demands, the masticatory muscle can adapt its fiber size and phenotypes.[28] The

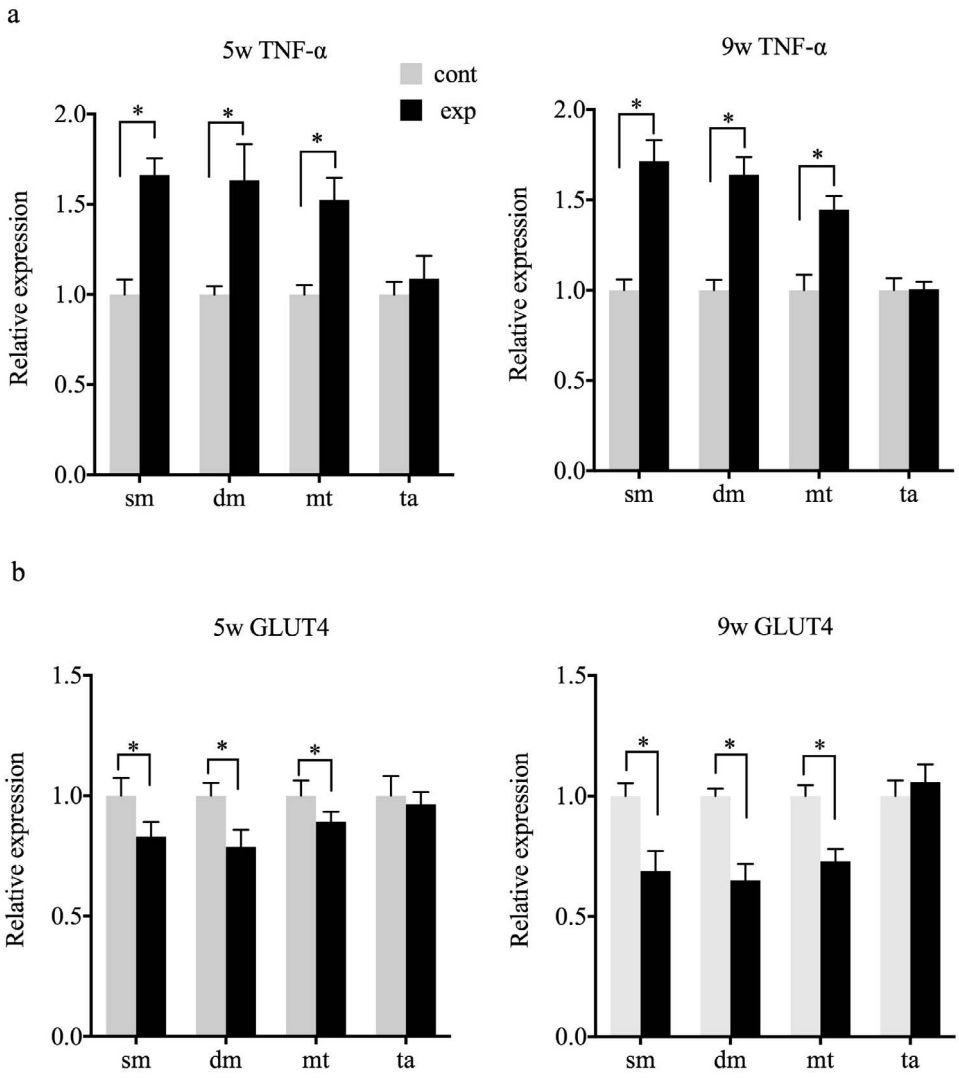

Figure 6. Messenger RNA expression of tumor necrosis factor-α (a) and glucose transporter 4 (b) in jaw-closing muscles. * $P < .05$. Abbreviations: Cont, control group; Exp, experimental group; sm, superficial masseter; dm, deep masseter; mt, medial temporalis; and ta, tibialis anterior.

morphologic changes of muscles can be caused by contraction, occlusal alteration, and mechanical overload.[29] It was reported that activity levels in masseter and anterior temporalis muscles among individuals who exhibit mouth breathing were lower at the maximum intercuspal position.[30]

The present study demonstrated that morphologic changes in the jaw-closing muscles occurred after nasal obstruction. These changes were in association with reductions in the CSA of the jaw-closing muscles, and a slow-to-fast-type change occurred in the masseter muscles, with a decrease in expression of MHC-2b and an increase in MHC-2a expression. According to the characteristics of MHC isoforms described previously[9] among fast fibers, compared with fibers expressing MHC-2a, those expressing MHC-2b generate faster shortening velocity, greater maximum specific force, and lower resistance to fatigue. The present investigation showed that unilateral nasal obstruction could reduce the contraction force of the masseter muscles.

In addition, the results showed that unilateral nasal obstruction increased expression of the protein and mRNA of TNF-α and decreased those of GLUT4 in jaw-closing muscles. TNF-α has long been recognized as a cytokine that reduces the contractile functions of skeletal muscles, and it is considered to be associated with cachexia as a mediator of muscle atrophy.[31,32] Li and Reid[13] concluded that the effect of TNF-α (ie, reduction in muscle contraction) could be prevented by pretreating muscles with the platelet activating factor receptor and an inhibitor of nitric oxide synthase. GLUT4 is the most important glucose carrier expressed in rat skeletal muscles.[33] Two signaling

pathways lead to GLUT4 expression, an insulin-activated signaling pathway and an insulin-independent signaling pathway that is activated by contraction.[34] Nasal obstruction may reduce GLUT4 expression via contractile dysfunction, thereby causing glucose dysregulation and muscle atrophy.

Numerous clinical studies have investigated the relationship between the function of masticatory muscles and the morphology of the craniofacial complex. There is a negative correlation between masseter muscle activity and occlusal force and the mandibular plane angle.[35] For example, patients with myotonic dystrophy who present with low activity in masseter muscles have abnormal bone development and a large mandibular plane angle.[36,37] The current results suggest that unilateral nasal obstruction can affect development of the mandible by reducing the growth and contraction activity of jaw-closing muscles. One of the disadvantages of a histochemical approach is the lack of information regarding dynamic changes in the tissues because only the static or fixed constituents were studied when the chemical tests were applied. The changes in MHC types of the jaw-closing muscles adapted to nasal obstruction in adult rats will continue to be observed after growth has been completed.

CONCLUSIONS

- Unilateral nasal obstruction in growing rats reduced the fiber CSA of the jaw-closing muscles and increased the percentage of MHC-2a in masseter muscles.
- After unilateral nasal obstruction, TNF-α expression was upregulated and that of GLUT4 downregulated in masseter and medial temporalis muscles compared with those in control group rats aged 5 and 9 weeks. These results suggest that unilateral nasal obstruction reduces the growth and contraction characteristics of jaw-closing muscles to affect craniofacial development.

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REFERENCES

1. Yamada T, Tanne K, Miyamoto K, Yamauchi K. Influences of nasal respiratory obstruction on craniofacial growth in young Macaca fuscata monkeys. *Am J Orthod Dentofacial Orthop*. 1997;111:38–43.
2. Kluemper GT, Vig PS, Vig KW. Nasorespiratory characteristics and craniofacial morphology. *Eur J Orthod*. 1995;17:491–495.
3. Harari D, Redlich M, Miri S, Hamud T, Gross M. The effect of mouth breathing versus nasal breathing on dentofacial and craniofacial development in orthodontic patients. *Laryngoscope*. 2010;120:2089–2093.
4. Gelhaye M, Martrette JM, Legrand-Frossi C, Trabalon M. Myosin heavy chain expression and muscle adaptation to chronic oral breathing in rat. *Respir Physiol Neurobiol*. 2006;154:443–452.
5. Scarano E, Ottaviani F, Di Girolamo S, Galli A, Deli R, Paludetti G. Relationship between chronic nasal obstruction and craniofacial growth: an experimental model. *Int J Pediatr Otorhinolaryngol*. 1998;45:125–131.
6. Hiyama S, Ono T, Ishiwata Y, Kuroda T, Ohyama K. Effects of experimental nasal obstruction on human masseter and suprahyoid muscle activities during sleep. *Angle Orthod*. 2003;73:151–157.
7. Tsai CY, Lin YC, Su B, Yang LY, Chiu WC. Masseter muscle fibre changes following reduction of masticatory function. *Int J Oral Maxillofac Surg*. 2012;41:394–399.
8. Bottinelli R, Canepari M, Pellegrino MA, Reggiani C. Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence. *J Physiol*. 1996;495:573–586.
9. Sieck GC, Regnier M. Invited review: plasticity and energetic demands of contraction in skeletal and cardiac muscle. *J Appl Physiol*. 2001;90:1158–1164.
10. Kawai N, Sano R, Korfage JA, et al. Adaptation of rat jaw muscle fibers in postnatal development with a different food consistency: an immunohistochemical and electromyographic study. *J Anat*. 2010;216:717–723.
11. Nandi J, Meguid MM, Inui A, et al. Central mechanisms involved with catabolism. *Curr Opin Clin Nutr Metab Care*. 2002;5:407–418.
12. Youd JM, Rattigan S, Clark MG. Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle in vivo by TNF-alpha. *Diabetes*. 2000;49:1904–1909.
13. Li YP, Reid MB. Effect of tumor necrosis factor-alpha on skeletal muscle metabolism. *Curr Opin Rheumatol*. 2001;13:483–487.
14. Garcia-Martinez C, Lopez-Soriano FJ, Argiles JM. Acute treatment with tumour necrosis factor-alpha induces changes in protein metabolism in rat skeletal muscle. *Mol Cell Biochem*. 1993;125:11–18.
15. Birnbaum MJ. Identification of a novel gene encoding an insulin-responsive glucose transporter protein. *Cell*. 1989;57:305–315.
16. Hoshino S, Sakamoto K, Vassilopoulos S, et al. The CHC22 clathrin-GLUT4 transport pathway contributes to skeletal muscle regeneration. *PLoS One*. 2013;8:e77787.
17. Uchima Koecklin KH, Kato C, Funaki Y, et al. Effect of unilateral nasal obstruction on tongue protrusion forces in growing rats. *J Appl Physiol*. 2015;118:1128–1135.
18. Carreras MC, Peralta JG, Converso DP, et al. Modulation of liver mitochondrial NOS is implicated in thyroid-dependent regulation of O_2 uptake. *Am J Physiol Heart Circ Physiol*. 2001;281:H2282–H2288.
19. Graziotti GH, Rios CM, Rivero JL. Evidence for three fast myosin heavy chain isoforms in type II skeletal muscle fibers in the adult llama (Lama glama). *J Histochem Cytochem*. 2001;49:1033–1044.
20. Oishi S, Shimizu Y, Hosomichi J, et al. Intermittent hypoxia influences alveolar bone proper microstructure via hypoxia-

inducible factor and VEGF expression in periodontal ligaments of growing rats. *Front Physiol*. 2016;7:416.
21. Afanador E, Yokozeki M, Oba Y, et al. Messenger RNA expression of periostin and twist transiently decrease by occlusal hypofunction in mouse periodontal ligament. *Arch Oral Biol*. 2005;50:1023–1031.
22. Niaki EA, Chalipa J, Taghipoor E. Evaluation of oxygen saturation by pulse-oximetry in mouth breathing patients. *Acta Med Iran*. 2010;48:9–11.
23. Van de Louw A, Cracco C, Cerf C, et al. Accuracy of pulse oximetry in the intensive care unit. *Intensive Care Med*. 2001;27:1606–1613.
24. Harvold EP, Tomer BS, Vargervik K, Chierici G. Primate experiments on oral respiration. *Am J Orthod*. 1981;79:359–372.
25. Levrini L, Mangano A, Ambrosoli A, Merlo P, Mangano C, Caprioglio A. Craniofacial growth and respiration: a study on an animal model. *Ann Stomatol*. 2015;6:53–57.
26. Deli R, Deli GUR. Nasal obstruction and craniofacial growth. *Eur J Orthod*. 1992;14:76.
27. Otani-Saito K, Ono T, Ishiwata Y, Kuroda T. Modulation of the stretch reflex of jaw-closing muscles in different modes and phases of respiration. *Angle Orthod*. 2001;71:201–209.
28. Goldspink G. The adaptation of muscle to a new functional length. In: Anderson D, Matthews B, eds. *Mastication*. Bristol, CT: John Wright and Sons; 1976:10.
29. Arai C, Ohnuki Y, Umeki D, Saeki Y. Effects of bite-opening and cyclosporin A on the mRNA levels of myosin heavy chain and the muscle mass in rat masseter. *Jpn J Physiol*. 2005;55:173–179.
30. Ferla A, Silva AM, Correa EC. Electrical activity of the anterior temporal and masseter muscles in mouth and nasal breathing children. *Braz J Otorhinolaryngol*. 2008;74:588–595.
31. Tracey KJ, Cerami A. Tumor necrosis factor: an updated review of its biology. *Crit Care Med*. 1993;21:S415–422.
32. Espat NJ, Copeland EM, Moldawer LL. Tumor necrosis factor and cachexia: a current perspective. *Surg Oncol*. 1994;3:255–262.
33. Marette A, Burdett E, Douen A, Vranic M, Klip A. Insulin induces the translocation of GLUT4 from a unique intracellular organelle to transverse tubules in rat skeletal muscle. *Diabetes*. 1992;41:1562–1569.
34. Jessen N, Djurhuus CB, Jorgensen JO, et al. Evidence against a role for insulin-signaling proteins PI 3-kinase and Akt in insulin resistance in human skeletal muscle induced by short-term GH infusion. *Am J Physiol Endocrinol Metab*. 2005;288:E194–199.
35. Ingervall B, Minder C. Correlation between maximum bite force and facial morphology in children. *Angle Orthod*. 1997;67:415–422.
36. Zanoteli E, Yamashita HK, Suzuki H, Oliveira AS, Gabbai AA. Temporomandibular joint and masticatory muscle involvement in myotonic dystrophy: a study by magnetic resonance imaging. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;94:262–271.
37. Yonemitsu I, Muramoto T, Soma K. The influence of masseter activity on rat mandibular growth. *Arch Oral Biol*. 2007;52:487–493.