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Title	Study of training for improving lip incompetence
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Abstract:

Purpose: We have been using myofunctional therapy in orthodontic treatment to improve orofacial disorders. Our previous study showed that lip training increased orbicularis oris muscle strength and endurance. The aim of this study was to determine the effectiveness of hypoxic lip training for improving lip incompetence.

Subjects & Methods: Twenty healthy subjects (10 males and 10 females, 23.6 ± 2.3 years old) with lip incompetence participated in this study. We recorded the sealed lip ratio calculated by using the formula "(lip-sealing time / total recorded time) × 100" during relaxation (listening to soothing music) and during concentration (performing a mathematical calculation). Then the subjects performed a standardized hypoxic lip training (5 repetitive contractions with 80% of maximum tensile strength of the orbicularis oris muscle) with a traction plate. Training was repeated daily for 4 weeks. To estimate training effects, the sealed lip ratios during relaxation and concentration were recorded before training (T1), at 2 weeks (T2) and 4 weeks (T3) after the start of training, and at 4 weeks (T4) and 8 weeks (T5) after the end of training.

Results: The sealed lip ratios in both the relaxation and concentration conditions significantly

(P < 0.003 after Bonferroni correction) increased during the training period. Although the sealed lip ratios slightly decreased during the post-training period, they were not significantly different from those at T3.

Conclusions: Hypoxic lip training increases the sealed lip ratio and is thus effective for improving lip incompetence. Sealed lip ratios were maintained after 8 weeks of training.

1. Introduction

In orthodontic treatment, myofunctional therapy (MFT) is used to improve orofacial disorders such as mouth breathing and abnormal tongue and lip resting postures. Coordination of the functions of the lips, cheeks, tongue, and jaws is essential in normal chewing and swallowing [1].

Lip competence is the condition whereby the lips are in light contact when the mandible is in its clinical rest position [2,3]. It has been reported that lip incompetence is associated with mouth breathing and ventilation disorders [4]. Lip incompetence has various negative effects leading to articulation defects, dysphagia, and periodontal disease as a consequence of dry mouth [5] and to the development of various types of malocclusion such as maxillary protrusion and open bite [6]. In contrast, lip competence has an

important role in the growth and development of the craniofacial complex [7]. Therefore, improving lip incompetence may help to resolve these problems.

There are several types of MFT training for improving lip incompetence including button pull exercises [8]. However, the effectiveness of these methods for improving lip incompetence must be estimated more objectively.

In the field of sports medicine, it has been reported that hypoxic muscle training, which is training under a condition that reduces the oxygen concentration in muscle, is effective for increasing muscle strength. On the other hand, aerobic exercise, which is training under a condition with a high oxygen concentration in muscle, is effective for increasing the endurance of muscles, which is persistence of muscle activity [9].

In a previous study, we measured oxygenation dynamics in the orbicularis oris muscle by using near-infrared spectrometry and evaluated training methods to improve the strength and endurance of the orbicularis oris muscle [10,11]. We revealed that aerobic training, which involved 20 repetitions with 50% of maximum tensile strength for the orbicularis oris muscle for 5 seconds and rest for 5 seconds, was effective for improving orbicularis oris muscle endurance. On the other hand, we showed that hypoxic training for the orbicularis oris muscle, which involved 5 repetitions with 80% of

maximum tensile strength for the orbicularis oris muscle for 5 seconds and rest for 5 seconds, was effective for improving both orbicularis oris muscle strength and endurance [11]. Ohtsuka reported that aerobic lip training, which increases orbicularis oris muscle endurance, improves lip incompetence in a patient with lip incompetence [12]. Guyton and Hall reported that muscles that contract at more than 50% of maximum force of contraction will rapidly develop muscle strength even if the contractions are performed only a few times each day [13]. Our previous study also showed that hypoxic training for the orbicularis oris muscle increases its strength.

The aim of this study was to determine the effectiveness of hypoxic orbicularis oris muscle training for improving lip incompetence.

2. Subjects and methods

2.1. Subjects

The subjects were 20 healthy Japanese volunteers (10 males and 10 females) who were undergraduate or graduate students of Hokkaido University with lip incompetence as defined in previous report. There have been various definitions of lip incompetence in past studies. In this study, we used the Satimary's definition based on cluster analysis [14]. Satimary reported that lip-incompetent patients had a sealed lip ratio

(percentage of lip-sealing time/total recorded time) of less than 30.1% at rest and less than 13.2% during a concentration task. These were the parameters by which we defined lip incompetence [14]. The mean age of the subjects was 23.6 ± 2.3 years (males: 24.3 ± 2.2 years, females: 22.9 ± 2.3 years). Exclusion criteria were any nasopharyngeal complaints, a previous history of orthodontic treatment, missing anterior teeth, any skeletal malocclusion (ANB<3.0° and 5.5° < ANB, using a non radiographic ANB angle measurement device [15]), and an abnormal overbite and/or overjet (~2.0 mm and 3.0 mm~).

2.2. Study design

This study was a single-arm exploratory study to investigate the effectiveness of hypoxic lip training for improving lip competence. To estimate the effectiveness of training, the orbicularis oris muscle strength and endurance and the lip sealing time during relaxation and concentration were recorded before training (T1) and 2 weeks (T2) and 4 weeks (T3) after hypoxic lip training started. Then to estimate the sustainability of the effect of training during the post-training period, muscle strength and endurance and lip sealing time were recorded at 4 weeks (T4) and 8 weeks (T5) after the termination of hypoxic lip training (Fig. 1). All of the measurement conditions (temperature, humidity, room lighting, period of the day in which measurements were conducted, and written instructions) were the same for all subjects.

2.3. Estimation of sealed lip ratio

A lip seal detection sensor (2.0 mm \times 5.0 mm \times 200 µm) was attached to the lower lip via a skin adhesive (Varicare®, Conva Tec, NJ, USA) in a position where the lower lip would come into contact with the upper lip (Fig. 2). Measurements recorded by a lip contact sensor and electrical recording device [16] were used for estimating the sealed lip ratio. The sealed lip ratio was calculated using the following formula: Sealed lip ratio = (lip sealing time/total recorded time) \times 100 [16]. The touch sensor was activated to flow electricity when the upper lip touches the sensor, and the signals were transferred as a waveform to the recorder (DL-2000, S&ME, Tokyo, Japan).

We calculated the sealed lip ratio in two situations: relaxation and concentration. During a period of relaxation, subjects listened to soothing music with their eyes closed for 10 min. During a period of concentration, subjects performed a mathematical 10×10 calculation using a free software package (10×10 calculation; Shimizu Takamitsu, Tokyo, Japan) for 10 min. At 5 min after each subject had started the relaxation task or concentration task, lip sealing time was recorded for 5 min.

The tasks were performed sequentially on the same day, in the same order, and consecutively with the subjects seated in an upright but relaxed position with their head unsupported and naturally oriented with noise canceling headphones (MDR-NC600D, Sony, Tokyo, Japan) in a sound attenuated laboratory.

2.4. Evaluation of orbicularis oris muscle strength

Traction plates (30 mm × 50mm × 3mm) (Fig. 3) were made of a thermoplastic material (Bioplast[®]; Scheu Dental GmbH, Iserlohn, Germany) [12]. Subjects were seated on a chair with their head and chin stabilized in a natural head position [17]. A traction plate was inserted in the upper and lower oral vestibules and the plate was pulled by the testing machine (Digital Force Gauge ZP & Motorized Test Stands MH-1000N; Imada, Toyohashi, Japan) via a string at a constant velocity of 45 mm/ min (Fig. 4). The subjects were instructed to bite with their molars and not suck on the plates. To prevent negative pressure, the plates had 12 holes each of 2 mm in diameter (Fig. 3). The tensile strength of the orbicularis oris was measured at the force required for the plate to come out of the oral vestibule. The subjects performed the test 3 times with 10-min rest intervals, and the maximum tensile strength value in the 3 measurements was used as maximum tensile strength (MTS) of the orbicularis oris muscle.

2.5. Evaluation of orbicularis oris muscle endurance

Subjects were seated on a chair with their head and chin stabilized in a natural head position. The traction plate was inserted into the upper and lower oral vestibules and the plate was pulled in a horizontal direction with a weight (equivalent to 80% of MTS) via a pulley (Fig. 5). The subjects were instructed to bite with their molars and not suck on the traction plates. We measured the time in seconds from the start of the test to the time when the traction plate came out of the mouth as endurance of the orbicularis oris muscle.

2.6. Hypoxic lip training

The traction plate was inserted into the upper and lower oral vestibules and connected to a weight (80% of MTS) by strings. The subjects stood and tipped their head forward and were instructed to bite with their molars and not suck on the plate. The training involved the weight being hung for 5 sec supported only by the lips and then supported in the hands for 5 sec performed 5 times following Ohtsuka's method (Fig. 6) [12]. The subjects performed this training daily for 4 weeks. If MTS had increased at 2 weeks after the start of training, a weight equivalent to 80% of the new value was used instead. We gave a checklist to each subject to check whether they had done the hypoxic lip training every day to keep their motivation to perform hypoxic lip training.

2.7. Statistical analysis

Differences in all measurements between T1, T2 and T3 (training period) were evaluated by one-way analysis of variance (ANOVA) with the post hoc Bonferroni test to estimate improvement of lip incompetence by hypoxic lip training. Differences in all of the measurement items between T3, T4 and T5 (post-training period) were also evaluated to estimate the sustainability of the effect of hypoxic lip training. The statistical analyses were performed with software (JMP ver.12; SAS Institute Inc., Cary, NC, USA) to determine statistical differences. Probability levels of P < 0.05 were considered statistically significant.

3. Results

Figures 7 and 8 show the orbicularis oris muscle strength and endurance at various measurement times, and Figure 9 shows the sealed lip ratios during relaxation and concentration.

3.1. Strength

Figure 7 shows that the strength increased gradually during the training period and decreased slightly during the post-training period. Strength value at T3 (1372.7 \pm 521.4 g) was significantly (p < 0.016 after Bonferroni correction) higher than that at T1 (981.7 \pm 338.2 g). Strength values during post-training at T4

and T5 (1368.1 \pm 559.3 g and 1344.0 \pm 598.6 g, respectively) were slightly smaller than the value at T3, but the difference was not significant.

3.2. Endurance

Figure 8 shows that endurance gradually increased during the training period and decreased during the post-training period. Endurance value at T3 (69.8 ±20.4 s) was significantly (T1: p < 0.003, T2: p < 0.016 after Bonferroni correction) higher than the values at T1 and T2 (41.7 ±17.0 s and 55.8 ± 20.1 s, respectively). Endurance values during the post-training period at T4 and T5 (64.1 ± 20.8 s and 59.4 ± 18.0 s, respectively) were smaller than that at T3, but the differences were not significant.

3.3. Sealed lip ratio

Figures 9A and 9B show the sealed lip ratios in the relaxation and concentration conditions. In both conditions, the sealed lip ratio increased during the training period and decreased during the post-training period. The sealed lip ratios during the two conditions at T2 ($64.6 \pm 30.6\%$ and $56.3 \pm 30.7\%$, respectively) and T3 ($93.3 \pm 10.1\%$ and $84.3 \pm 21.3\%$) were significantly (p < 0.003 after Bonferroni correction) larger than those at T1 ($12.5 \pm 10.1\%$ and $7.0 \pm 4.1\%$), and those at T3 were significantly (p < 0.003 after Bonferroni correction) larger than those at T2. The sealed lip ratios during post-training at T4 ($89.6 \pm 10.1\%$) and $7.0 \pm 10.1\%$ and 7

18.1% and 78.7 \pm 19.3%) and at T5 (84.8 \pm 20.6% and 70.6 \pm 27.9%) were smaller than those at T3, but the differences were not significant.

4. Discussion

4.1. Subjects

Many orthodontists think that lip incompetence causes malocclusion and that skeletal problems also cause incomplete lip sealing. In this study, we chose specific subjects who had lip incompetence but no malocclusion, because we wanted to focus on the functional problems. We found that hypoxic training of the orbicularis oris muscle was effective for subjects who had only functional problems.

4.2. Effectiveness of training

It has been reported that hypoxic muscle training is effective for improving muscle strength and that aerobic muscle training is effective for improving muscle endurance [9]. On the other hand, Kon et al. showed that muscular endurance was significantly increased under hypoxic exercise training in human skeletal muscle [18]. Consistent with their results, the results of this study showed that hypoxic lip training was effective for increasing both orbicularis oris muscular strength and endurance because both of them were greatly increased after the hypoxic training at T3 (Figs.7 and 8). The results of this research also showed that hypoxic lip training significantly increased the sealed lip ratio. When we investigated data for each subject, there were no subjects whose strength and endurance and sealed lip ratio at T3 were smaller than those at T1.

The orbicularis oris muscle consists of 71.8% type IIa muscle fibers, which are fast-twitch fibers suited for instantaneous exercise related to muscle strength. The muscle also consists of 28.2% type I muscle fibers, which are slow-twitch fibers suited for aerobic exercise related to muscle endurance [19,20].

The results of this study indicate two possible reasons for the increase in sealed lip ratio with hypoxic orbicular oris muscular training. One possible reason is reinforcement of type I muscle fibers. In a previous study, Ohtsuka revealed that aerobic lip training actually increases the sealed lip ratio and that the aerobic training also increases orbicular oris muscular endurance rather than muscular strength. It can be assumed that the increased orbicular oris muscular endurance, which was induced even with the hypoxic training in the present study, resulted in improvement of lip incompetence [12].

The other possible reason for the increased sealed lip ratio by hypoxic muscle training might be reinforcement of type IIa muscle fibers. Although it has been clarified that muscular endurance ability is related to lip sealing ability, the interrelation between orbicularis oris muscular strength ability and lip sealing ability has not been clarified yet. A possible reason for the increase in sealed lip ratio with hypoxic orbicularis oris muscle training is that the increase in muscular strength ability of the orbicular oris muscle directly causes an increase in sealed lip ratio.

The interrelation between lip sealing ability and orbicular oris muscular strength is still not clear and further study is needed.

Although the reason is not clear, it was revealed in this study that hypoxic lip training improves lip incompetence as does aerobic lip training.

4.3. Post-training period

There were slight decreases in strength, endurance and sealed lip ratio during the post-training period, but the differences were not significant. It was shown in this study that orbicularis oris muscle strength, endurance, and lip competence were maintained at 8 weeks after the end of hypoxic lip training. Santos et al. reported that no significant changes in muscle endurance ability were observed following a 12-week detraining period after an 8-week period of resistance training [21]. Some studies have shown that detraining causes a decrease in muscle activities [22-24], but retraining results in a rapid return to the previously trained situation [25]. Further study is needed to determine how long the acquired muscular ability can maintain a lip-competent situation.

5. Conclusion

In this study, we investigated the effect of hypoxic lip training on sealed lip ratio in subjects with lip incompetence. Hypoxic lip training increases the sealed lip ratio and is thus effective for improving lip incompetence. The sealed lip ratio slightly decreased but was maintained for 8 weeks after the termination of training.

Ethical approval

All subjects signed informed consent forms before the start of this study, and the research protocol was approved by the Ethics Review Committee for Clinical and Epidemiological Study, Graduate School of Dental Medicine, Hokkaido University (approval no. 9 [2015]).

Conflict of interest

All authors have no conflict of interest in this study.

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Legends to Figures

Fig. 1. Study design. T1, start of hypoxic lip training; T2, 2 weeks after the start of training; T3, 4 weeks after the start of training; T4, 4 weeks after the end of training; T5, 8 weeks after the end of training.

Fig. 2. Evaluation of sealed lip ratio. A lip seal detection sensor $(2.0 \text{ mm} \times 5.0 \text{ mm} \times 200 \text{ }\mu\text{m})$ was attached to the lower lip via a skin adhesive in a position where the lower lip would come into contact with the upper lip.

Fig. 3. Traction plate.

Fig. 4. Evaluation of orbicularis oris muscle strength.

Fig. 5. Evaluation of orbicularis oris muscle endurance.

Fig. 6. Method of hypoxic lip training. The training involved a weight being hung for 5 sec supported only by the lips and then supported in the hands for 5 sec performed 5 times.

Fig. 7. Change in strength of the orbicularis oris muscle (mean \pm standard deviation). *: p < 0.016 after Bonferroni correction.

Fig. 8. Change in endurance of the orbicularis oris muscle (mean \pm standard deviation). *: p < 0.016, **: p < 0.003 after Bonferroni correction.

Fig. 9. Results (mean \pm standard deviation) of evaluation of the sealed lip ratio during a relaxation

task (A) and during a concentration task (B). **: p <0.003 after Bonferroni correction.

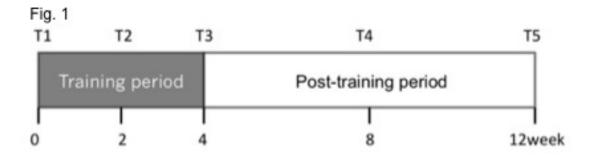


Fig. 2









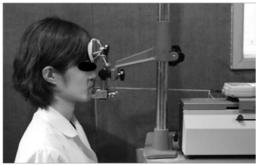


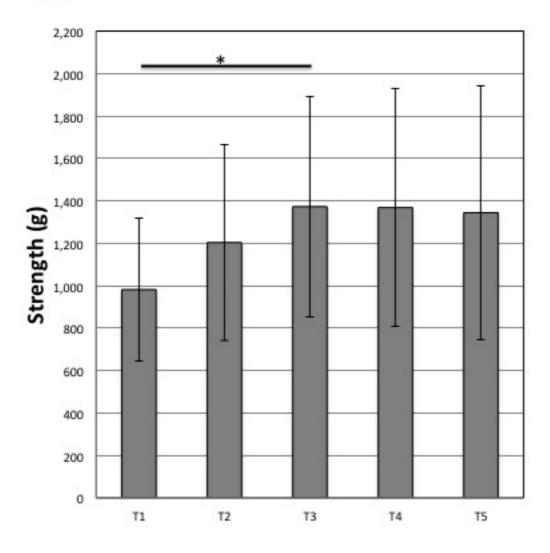


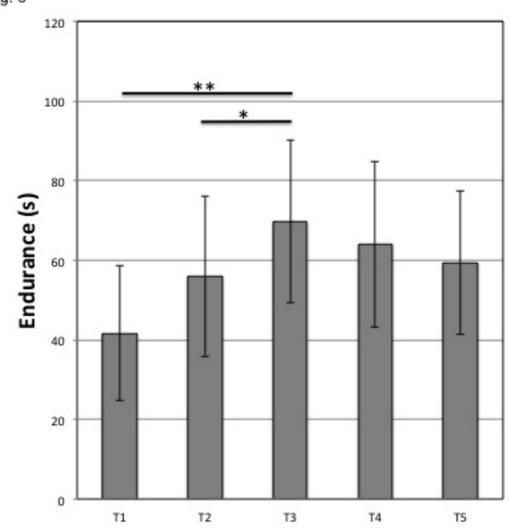


Fig. 6









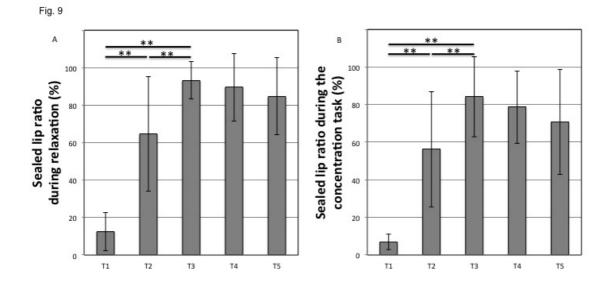


Fig. 8