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ORIGINAL ARTICLE

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Quantification of oxidative metabolism in masseter muscle of denture wearers

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Abstract This study aimed to quantify oxidative metabolism in masseter muscle using near-infrared spectroscopy, in particular for denture wearers. Fourteen normal dentate subjects without malocclusion (ND group, 25–50 years) participated in the quantification of oxidative metabolism. Eleven partially edentulous patients without occlusal stops (PD group, 64–80 years) and ten edentulous patients (CD group, 57–84 years) also participated after prosthodontic treatment. Oxidative metabolism was recorded during gum chewing, maximum clenching and regulated clenching at 5 kgf. The oxygenated hemoglobin at 5 kgf clenching level was normalized to the oxygenated hemoglobin at the lowest blood flow and expressed as oxygen consumption rate (OCR). The relationship of the OCR to the maximum clenching force was analyzed using Pearson's correlation coefficient, and differences between the PD and CD groups were tested by unpaired Student's *t*-test. The OCR showed a significant negative correlation with maximum clenching force in the ND group. The OCR of the PD group was significantly greater than that of the CD group, although the difference in maximum clenching force was not significant between both groups. These results suggest that the aerobic ability of masseter muscle in complete denture wearers is relatively greater than in partial denture wearers with same age level.

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Introduction

Near-infrared spectroscopy (NIRS) is a useful method for evaluating relative changes in the oxygenation and the deoxygenation of hemoglobin in human tissues [1–3]. In the study of skeletal muscles, Hamaoka et al [2] studied the relationship between oxygen consumption in the muscle (measured using NIRS) and high-energy substances in the muscle, for example phosphocreatine (PCr) and adenosine diphosphate (ADP) during hand-grip exercise, and reported that NIRS had the ability to evaluate the metabolic ability of muscle. Chance et al [3] found that the recovery time of reoxygenation in muscle after exercise reflected the balance between supply and consumption of the oxygen in muscle, and this could be used to evaluate the aerobic ability of the muscle. The above-mentioned reports suggest that the metabolic and aerobic ability of muscle can be evaluated using NIRS. In the field of dental science, Delcanho et al [4] reported that a subject with chronic pain in the craniomandibular muscles had the same level of maximum clenching force but a lower level of oxygenation of masseter muscle compared to healthy subjects, and suggested that there was a relationship between muscle pain and changes in oxidative metabolism.

Some studies have shown that the provision of complete denture has a positive effect on physical functions as well as oral ones. Fujinami et al [5] studied changes in gait, body sway and contributions to postural control caused by wearing the new denture. Occlusal rehabilitation, such as that caused by using a complete denture, also accelerates muscle function. Tallgren and Tryde [6] studied the muscle activity in 27 patients with upper complete and lower partial dentures and reported that the activity of jaw-closing muscle during chewing was normalized after six months of denture delivery and muscle intensity was maintained two years from the re-establishment of posterior occlusion using a removable denture. This acceleration of muscle function should influence the circulatory system in the brain. Sasaki [7] demonstrated that cerebral flow in the frontal region was increased by gum chewing. From these reports, it is possible to speculate that an acceleration of muscle function would contribute positively to the circulatory system of the craniomandibular region. However, an analysis of the oxidative metabolism and the aerobic ability in jaw-closing muscle has not been performed yet, and the relationship of oxidative metabolism to occlusal state and masticatory performance is yet to be studied. The aim of this study was to quantify the oxidative metabolism in masseter muscle using NIRS, and to investigate the oxidative metabolism in complete denture wearers.

Table 1 Gender, age, and occlusal state in two groups of denture wearers

Parameter	PD group (n=11)	CD group (n=10)
Gender	7 males, 4 females	5 males, 5 females
Age (years)	64–80, average 73.8	57–84, average 71.4
Number of remaining teeth[a]	Upper 2.7, lower 4.9	Upper 0, lower 0
Number of occlusal contacts[a]	Anterior 0, posterior 0	Anterior 0, posterior 0
Denture type	Removable partial denture or overdenture	Complete denture

PD group Partial denture wearers, *CD group* complete denture wearers

[a]Data are presented as means

Materials and methods

Subjects

Fourteen normal dentate subjects (ND group) (six males and eight females), aged from 25 to 50 years, participated in the quantification of oxidative metabolism in masseter muscle. The inclusion criteria for the ND group were continuous dental arches without occlusal contact on the third molars, and no restorations covering the entire occlusal surfaces of the teeth. The relationship of oral rehabilitation to oxidative metabolism was then investigated in 21 elderly denture wearers who had complained of impaired chewing function due to tooth loss, had visited the dental hospital of Tokyo Medical and Dental University and were satisfied with the improved function provided by new dentures or by adjustment of their current dentures. Denture wearers were divided into two groups according to their occlusal state for control trials; 11 partially edentulous patients without occlusal stops (PD group) (seven males and four females) who were aged from 64 to 80 years and were provided with removable partial dentures or overdentures, and ten edentulous patients (CD group) (five males and five females) who were aged from 57 to 84 years and were provided with complete dentures. Gender, age and occlusal state details for the PD and CD groups are shown in Table 1. None of them had any significant medical problems or signs or symptoms of craniomandibular disorders. Age, gender, body height and weight were recorded by questionnaire for all subjects. Informed consent was obtained from each subject before the study. This study was reviewed and approved by the Ethics Review Committee of Tokyo Medical and Dental University.

Maximum clenching force and masseter muscle thickness

The bite force during maximum clenching for 3 s was recorded five times, with intervals of about 1 min, on pressure sensitive film (Fig. 1, Dental Prescale 50HR; Fuji Film, Tokyo) using one film per clench. The five recorded films from each subject were analyzed using a computerized scanner (Occluzer FPD-705, Fuji Film, Tokyo) and the median was set as the maximum clenching force.

The origin, insertion, anterior border and posterior border of the right masseter muscle were marked by palpation and the center of the muscle was shown in all subjects. Five cross-sectional images of the right masseter muscle during the rest position was recorded using ultrasonography (EUB-6000, Hitachi Medico, Tokyo) with a 13.0-MHz/38-mm linear-array transducer. The distance from the fascia to the ramus was measured at the center of the muscle on the monitor. The muscle thickness was calculated as the mean of the five measurements.

Recording the oxidative metabolism

Changes in oxygenated hemoglobin in the masseter muscle were recorded by the NIRS system (OM-220, Shimadzu, Kyoto), which consisted of a computerized control apparatus, a monitor and two probes. The probe used in this study was placed on the muscle and has one light source and two detectors at a distance of 2.5 cm (Fig. 2). Light with two wavelengths (780 and 830 nm) are emitted alternately from the light source. A silicon photodiode was used as the light detector. All data were automatically stored every second and saved onto a floppy disk. Each subject sat on a dental chair in an upright position with his/her head on the headrest. The silicone cover was arranged to in order to prevent light leakage from the probe and excessive pressure on the masseter muscle and its surrounding tissue. After marking the masseter muscle by palpation, the probe and silicone cover was placed on the middle of the right and the left masseter muscle. The subjects were asked to perform the following tasks:

(1) Regulated clenching for 10 s at 5 kgf with an Occlusal Force Meter (GM10, Nagano Keiki, Tokyo; measurement range: 0–100 kgf, measurement accuracy: ±1 kgf) placed on the first molar of the mandible on the habitual chewing side.

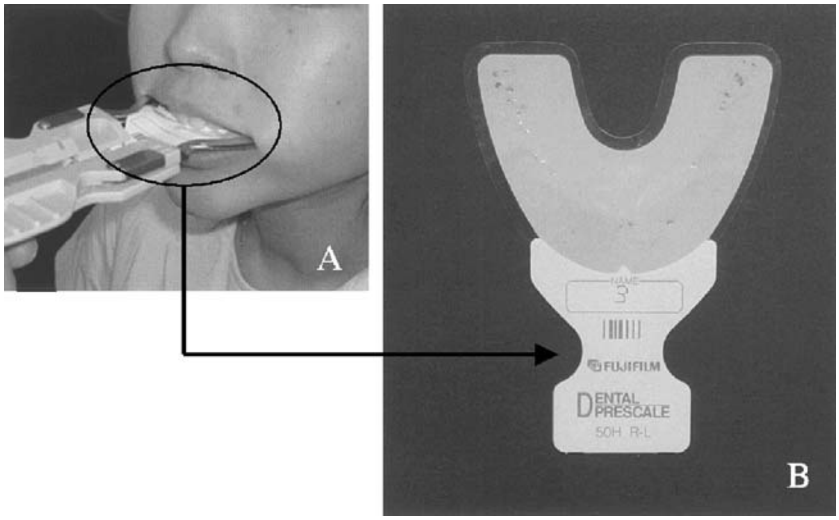

Fig. 1A–B Clenching force was recorded with a horseshoe-shaped pressure sensitive film of 0.1 mm thickness inserted between the upper and lower dental arches (**A**). Applying load turns the white film various shades of red (**B**). The computerized scanning apparatus counts the number of red dots and indicates the shade of red. The number of dots and the shades are converted to the area (mm^2) and the pressure (MPa), respectively, and the force (N) is automatically calculated by multiplying the area by the pressure

(2) Gum chewing (Freezone, Lotte, Tokyo) for 1 min on the habitual chewing side at a rate of 65 times per minute.
(3) Maximum voluntary clenching in intercuspal position or centric occlusion for 10 s.

All measurements were performed three times at >1 min intervals until the oxygenation levels recovered to resting levels.

Analysis of oxygenated hemoglobin

The influence of the fat layer was corrected by substituting the thickness of the fat layer (measured using ultrasonography) into the formula reported by Niwayama et al [8]. Typical changes in oxygenated hemoglobin (oxy-Hb) are shown in Fig. 3. The oxy-Hb decreased rapidly following the start of muscle contraction and reached a minimum level at which the oxygen supply and consumption was balanced. After the cessation of clenching or gum chew-

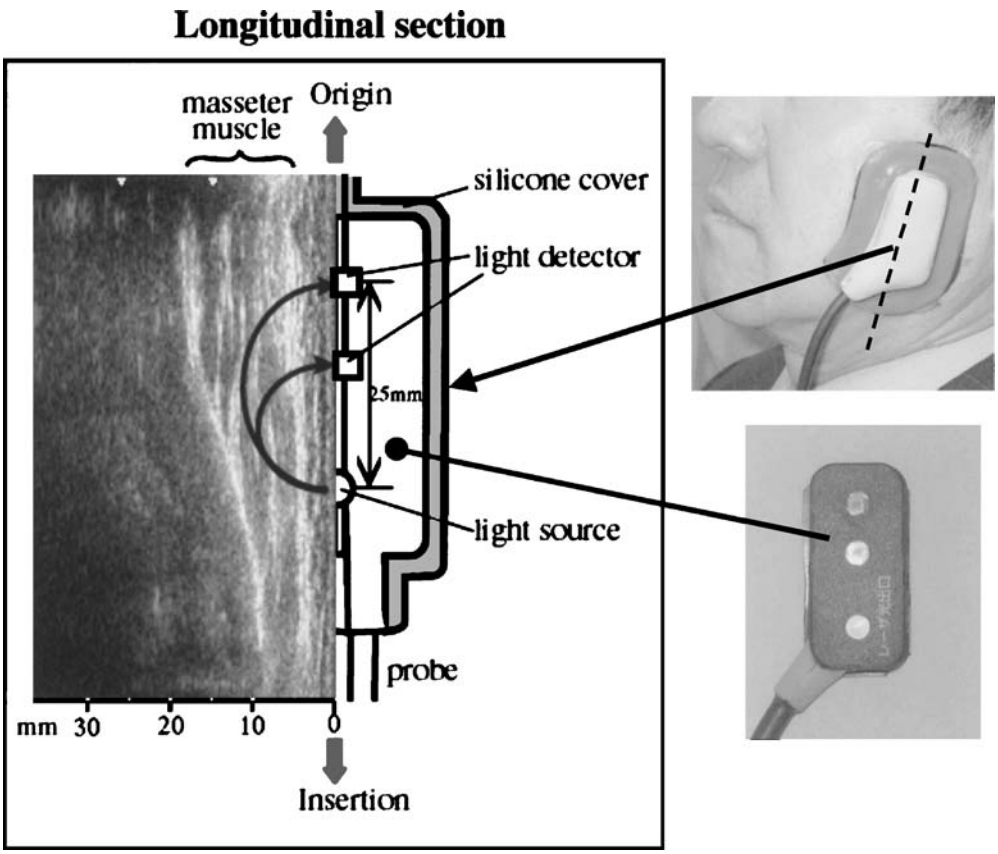

Fig. 2 The probe with silicone cover was placed on the masseter muscle by adhesive tape, with the light source and detectors aligned parallel to the masseter muscle fibers. The position of the probe on the masseter muscle is illustrated schematically using an ultrasound image

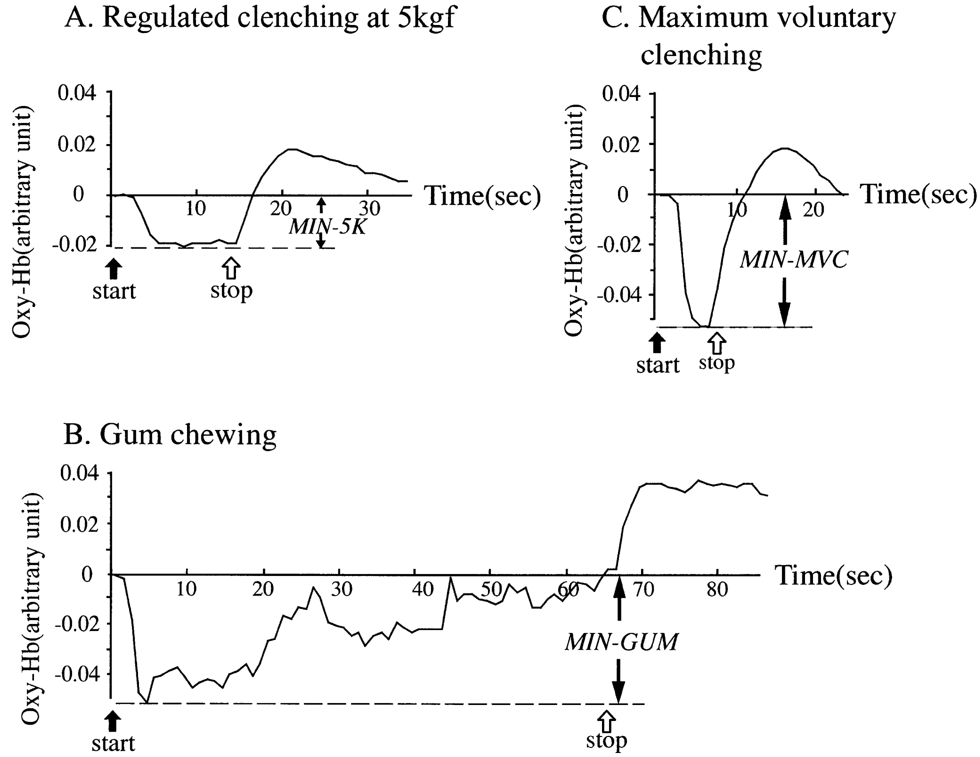

Fig. 3 Changes in oxygenated hemoglobin are shown during regulated clenching at 5 kgf (*upper left*), gum chewing (*lower*) and maximum voluntary clenching (*upper right*). The changes are expressed in arbitrary units

Oxygen Consumption Rate (OCR) = *MIN-5K* / *MIN-OXYHB* × 100

ing, the oxy-Hb increased rapidly due to reactive hyperemia. The minimum values during regulated clenching at 5 kgf, gum chewing and maximum voluntary clenching were defined as MIN-5k, MIN-GUM and MIN-MVC, respectively. MIN-OXYHB was defined as a larger absolute value within MIN-GUM or MIN-MVC. The ratio of MIN-5k to MIN-OXYHB was calculated and defined as the oxygen consumption rate (OCR).

Statistical analysis

As there were no significant gender differences in OCR, maximum clenching force and masseter muscle thickness, the male and female data were pooled for the following analysis.

(1) Differences in oxy-Hb between the MIN-5k, MIN-GUM and MIN-MVC were examined in each of the ND, PD, and CD groups by one-way analysis of variance (ANOVA).
(2) Correlations between age, OCR, maximum clenching force, masseter muscle thickness, body weight and height in the ND group were analyzed by Pearson's correlation analysis.
(3) Differences between the PD and the CD groups were examined for age, OCR, maximum clenching force, masseter muscle thickness by unpaired t-test.

P values were two-tailed with a significance level of 5%. Calculations were performed using statistical software (SPSS V.10.0J, Tokyo).

Results

The mean value of MIN-5k was significantly smaller than that of MIN-OXYHB in the ND group. There were no significant differences between MIN-GUM and MIN-MVC

Table 2 Means and standard deviations of MIN-5k, MIN-GUM, MIN-MVC and MIN-OXYHB in the ND, PD and CD groups

	ND group (n=14)	PD group (n=11)	CD group (n=10)
MIN-5k	0.013±0.009*	0.014±0.007	0.007±0.006
MIN-GUM	0.032±0.022*	0.018±0.008	0.014±0.007
MIN-MVC	0.034±0.029*	0.014±0.008	0.006±0.003
MIN- OXYHB	0.039±0.028*	0.020±0.009	0.014±0.007

Differences between values were tested in each group
MIN-5k Minimum value of oxy-Hb during regulated clenching at 5 kgf, *MIN-GUM* minimum value of oxy-Hb during gum chewing, *MIN-MVC* minimum value of oxy-Hb during maximum voluntary clenching, *MIN-OXYHB* greatest absolute value within MIN-GUM or MIN-MVC, *ND group* normal dentate subject, *PD group* partial denture wearers, *CD group* complete denture wearers
*P<0.05. Each value is in arbitrary units

Table 3 Correlation coefficients among age, OCR, maximum clenching force (MCF), masseter muscle thickness (MMT), body weight and height in the ND group

	Age	OCR	MCF	MMT	Body weight
OCR	−0.08				
MCF	0.08	−0.58*			
MMT	0.31	−0.28	0.56*		
Body weight	0.08	−0.18	0.62*	0.58*	
Body height	−0.33	−0.14	0.37	0.35	0.81**

OCR Oxygen consumption rate, *ND group* normal dentate subject
*P<0.05
**P<0.01

Table 4 Means and standard deviations of age, OCR, maximum clenching force (MCF), masseter muscle thickness (MMT) in the ND, PD and CD groups

	Age (Y)	OCR	MCF (N)	MMT (mm)
NDgroup (n=14)	31.9±9.0	39.7±18.9	1,384.8±528.1	7.8±2.2
	Age (Y)	OCR	MCF (N)	MMT (mm)
PD group (n=11)	73.8±6.6	71.5±17.4*	508.5±239.7	7.6±1.4
CD group (n=10)	71.4±8.7	52.0±4.5*	436.1±191.5	8.5±2.0

Differences were tested between the PD and CD groups
OCR Oxygen consumption rate, *ND group* normal dentate subject, *PD group* partial denture wearers, *CD group* complete denture wearers
*P<0.05. OCR is in arbitrary units

in the three groups, but the MIN-GUM in the PD and CD groups was greater than the MIN-MVC (Table 2). The OCR showed a significant negative correlation with maximum clenching force (r=−0.58), but no significant correlations with masseter muscle thickness, body weight and height in the ND group (Table 3). A scattering diagram was used to study the relationship between the OCR and the maximum clenching force in the ND group (Fig. 4). The OCR in the PD group was significantly greater than that in the CD group (P<0.05); however the values of age, maximum clenching force and masseter muscle thickness did not differ significantly between the two groups (Table 4).

Discussion

The distance between the light source and the detector in the LED probe used in this study was 25 mm, and the mean penetration depth from the surface of the probe was estimated to be about 10–12.5 mm for the 25-mm separation [9]. The thickness of the masseter muscle and subcutaneous tissue including the skin and the fat layer were also measured using ultrasonography and was 7.8±2.2 and 3.7±1.3 mm in the ND group, and 8.0±1.7 and 4.2±1.7 mm in the denture wearers, respectively. These findings certified that the infrared light was definitely absorbed and reflected in the masseter muscle and received by the detector.

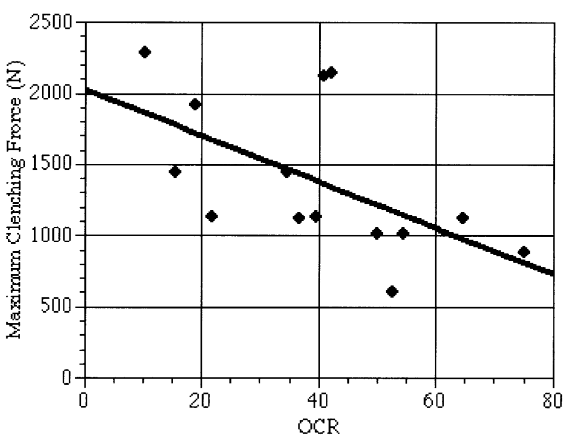

Fig. 4 The relation between the OCR and the maximum clenching force in the ND group. The OCR in the ND group showed a negative correlation with maximum clenching force

Biochemical analysis with a biopsy specimen, where the intramuscular phosphate compounds and other metabolic products were observed [10] and the indicator dilution method was used to measure local blood dynamics [11], has been used previously for metabolic measurements of local skeletal muscle. However, these methods required an invasive procedure and were limited in clinical application. Furthermore, they were unsuitable for continuous recording. NIRS permits continuous and noninvasive recording, and its clinical use has been rapidly spreading since the report by Jöbsis in 1977 [12]. On the other hand, regarding the measurement of oxidative metabolism, NIRS has a theoretical limitation in so far as the level of oxygenated and deoxygenated hemoglobin cannot be assessed absolutely. In the Beer–Lambert law, the absorption rate is proportional to the concentration of the material transmitting the light and the optical pathlength. The incident light is reflected and scattered because the vital tissue is not homogeneous and this results in a complicated pathway. This extreme difficulty with measuring the exact pathlength causes the methodological limitation. In order to solve this problem, Chance et al [3] measured the recovery time of the oxygenated and deoxygenated hemoglobin after exercise, and Hamaoka et al [2] interrupted the arterial blood flow using a tourniquet cuff and defined the minimum level during cuff ischemia as 0% and the hemoglobin level before interrupting the blood flow as 100% in order to normalize the oxygenated and deoxygenated hemoglobin levels. However, the technique of interrupting the arterial blood flow cannot be applied to a masseter muscle. De Blasi et al [13] reported that the oxygen consumption in forearm brachio-radial muscle during maximum voluntary contraction was not significantly different between the times when the blood flow was limited and when it wasn't. Ohkubo et al [14] reported that the blood volume in the trapezium muscle could be evaluated by measuring the change in blood volume normalized to the hyperemic peak value following isometric maximum voluntary contraction. The above-mentioned reports suggested that normalizing the oxygenation level in maximum voluntary contraction was a useful method for evaluating oxygen metabolism in muscle to which the technique of cuff occlusion could not be applied. Then, the oxygenated hemoglobin in the masseter muscle at the regulated clenching level (5 kgf) was normalized to oxygenated hemoglobin at the lowest blood flow and newly expressed as the oxygen consumption rate (OCR). In reports that examined the relationship between the contraction level of skeletal muscle and the blood flow, Humphreys and Lind [15] reported that the blood flow in the forearm muscle was interrupted with a tension of 70% MVC. Barcroft and Millen [16] found that the plantar flexors of the foot were almost ischemic during contraction with 20% MVC. In addition, Bonde-Peterson et al [17] reported that the blood flow in elbow extensors, elbow flexors and back extensors almost stopped at 25, 50 and 40% of MVC, respectively. Thus, interruption of the blood flow is induced above a certain level of muscle contraction, although the levels are different in each muscle. In the present study, no significant differences were observed between MIN-GUM and MIN-MVC in any groups, so we may predict that the blood flow would be interrupted during gum chewing at the same level as maximum clenching. The determination of the minimum value was very important for the normalization. Maximum clenching was an unexpectedly hard task for the elderly denture wearers in this study and the oxygenated hemoglobin level during maximum clenching was dramatically smaller than the level during gum chewing in more than half of the elderly denture wearers (PD group, nine out of 11 subjects; CD group, nine out of ten subjects), whereas this was six out of 14 in the ND group. The oxygenated hemoglobin in gum chewing was regarded as being equivalent to that in maximum voluntary contraction in those cases, and used as the standard for normalizing the oxygenation level.

The changes in the oxygenation levels observed in this study can be explained by the balance between oxygen consumption and supply in the tissue. The muscle oxygenation level decreased following the start of exercise because oxygen consumption exceeded oxygen supply to produce energy [1–3]. When oxygen consumption and supply were well balanced, the muscle oxygenation level was constant and rapidly increased after the cessation of exercise due to reactive hyperemia. Kimura et al [18] suggested that the low oxygenation level observed at the beginning of exercise reflected the energy necessary for muscle contraction. These results showed the significant negative correlation between OCR and maximum clenching force in the ND group. Generally, training will not only cause morphological changes in the skeletal muscle, like hypertrophy of muscle fiber [19], but also circulatory changes, like increased capillary vessel and well blood supply to each muscle fiber [20]. The ability of oxygen intake will therefore be greatly improved by training. The thickness of the masseter muscle had a positive relationship with the maximum clenching force, and a better aerobic ability was expected in the masseter muscle of a subject with stronger clenching force. As the changes in oxygenation level indicated the balance of oxygen consumption and supply, it was estimated that the oxygen supply was greater than the oxygen consumption in the subject with stronger clenching force, and the negative relation between maximum clenching force and the OCR in this result would support this estimation. On the other hand, chewing movement was performed by the contraction and relaxation of masticatory muscles, and seemed to be an aerobic exercise with low intensity and long duration, differing from maximum voluntary clenching in terms of energy production. OCR suggests that the aerobic ability of the masseter muscle during energy production is related to the chewing movement.

Our findings of no significant difference in maximum clenching force between the PD and CD groups was the same as found in previous studies. Yamashita et al [21] compared maximum bite force and masticatory performance in the subjects with removable partial dentures, complete dentures and normal dentitions and found that the maximum bite force and masticatory performance in the denture wearers with functional units were significantly greater than those of the complete denture wearers, but that those in the denture wearers without functional units were not significantly different from those of complete denture wearers. This suggests that the presence of the functional tooth units was the key factor in masticatory function. On the other hand, the results from the OCR in this study showed different findings: that the OCR of the PD group was significantly greater than that of the CD or ND groups. This suggests that the OCR does not chart the stability of occlusion, and the aerobic ability of masseter muscle in the PD group was lower than that of the CD and ND groups. Garrett et al [22] recorded the EMG activity of masseter muscle when chewing carrots and peanuts in 21 denture wearers with a mean age of 67.7 years in order to study the changes in muscle activity resulting from improvements in poorly-fitting dentures, and reported that a significant decrease in masseter activity was found after improving poorly-fitting dentures. Moreover, in another study that investigated masticatory performance with the same subjects [23], they reported that the duration of one chewing cycle became shorter and the time taken to swallow was shortened after the improvement of their dentures. Gunne [24] periodically investigated the masticatory performance and dietary intake of 19 patients (mean age 58.4 years) with removable partial dentures in their mandibles and reported that the masticatory efficiency improved, but diet preference did not change after denture provision. The same findings were obtained for complete denture wearers [25]. The diet preferences of elderly denture wearers may be greatly affected by age rather than occlusal factors. Therefore, the diet preferences of the PD and CD groups should be the same in this study because both groups were almost the same age. From these previous reports, and noting the hypothesis that removable partial dentures are normally more stable than complete dentures, it may be suggested that the lower chewing ability of the CD group would be compensated by the greater muscle activity, the greater number of chewing strokes, and/or the longer period of time until swallowing compared to that in the PD group. The smaller OCR, suggesting a higher aerobic ability, of the CD group compared to the PD group may result from the different chewing styles of both groups.

Conclusion

This study is the first report where the oxidative metabolism in masseter muscle was monitored in order to evaluate masticatory function (as far as the authors know), and results suggest that the oxygen consumption rate (OCR), which was used to quantify oxygen metabolism in masseter muscle, can be used to monitor the aerobic ability of masseter muscle and evaluate the frequency and intensity of chewing movement. Further research into its relationship to masticatory performance, chewing movement and occlusal state is necessary in order to be able to use the OCR to evaluate occlusal rehabilitation by prosthodontic treatment.

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