

ORIGINAL ARTICLE

Oxygen saturation and electromyographic changes in masseter muscle during experimental chewing of gum with harder texture

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Abstract

Objective. The purpose of this study was to clarify the relationship between changes in masseter muscle oxygenation measured by near-infrared spectroscopy (NIRS) and changes in the electromyographic (EMG) power spectrum during experimental chewing of gum with harder texture, to improve the understanding of the use of NIRS in assessing masseter muscle fatigue. **Material and methods.** Ten female volunteers with normal occlusion were examined. Mean age (standard deviation) was 28.4 (3.8) years. Mean fracture stress of gum was $12.5 \times 10^4 \text{ N/m}^2$. Subjects were instructed to chew gum for 60 s (75 strokes) on the voluntary chewing side at a pace of 1.25 strokes/s. Simultaneous recordings of NIRS and EMG signals from masseter muscle were performed during gum chewing. **Results.** Oxygen saturation levels decreased from the start of chewing, then stabilized with a break point between the two phases. The normalized EMG amplitude increased and the mean frequency of the EMG power spectrum decreased during gum chewing. The timing of break point appearance was related to the timing of a significant decrease in median frequency, but no clear relationships were found between break point appearance and increased EMG amplitude. **Conclusions.** These results suggest that the break point of the oxygen saturation curve, as obtained from NIRS measurements, could be used as an indicator of masseter muscle fatigue as assessed by a shift in the EMG power spectrum to lower frequencies.

Key Words: EMG, masseter muscle fatigue, median frequency, near-infrared spectroscopy (NIRS), oxygen saturation

Introduction

Fatigue of the masticatory muscles is thought to be related to chewing food with harder texture [1,2] and masticatory myofascial pain [2–5]. When hard foods are encountered in daily life, feelings of tiredness around the jaw and difficulty in continuing chewing may arise [1,2]. The degree of complaint seems to be related to individual differences in the ability to masticate and the type of occlusion. Studies have suggested that masticatory myofascial pain with temporomandibular dysfunction accelerates fatigue of masticatory muscles [4] and disturbs recovery from the fatigue [2]. Tortopidis et al. [5] reported that fatigue resistance of the masseter muscles was reduced in patients with temporomandibular disorder compared with healthy subjects.

Muscle fatigue has been widely studied in the limbs. Many studies have shown that the power

spectrum from electromyographic (EMG) signals shifts toward lower frequencies during fatiguing contractions [6–11]. For the masticatory muscles, several studies [12–15] have shown shifts in the spectrum toward lower frequencies during maximal or sub-maximal clenching. Lyons et al. [14] found a close relationship of median frequency shift to the subjective perception of fatigue as measured on a visual analog scale in the anterior temporal and masseter muscles during sustained isometric contractions. The frequency shift is due to slowing of the conduction velocity of action potentials along the muscle fibers and, therefore, to increased duration of the motor unit action potential [6,7,9,10].

Recently, near-infrared spectroscopy (NIRS) has been applied to monitor blood flow changes and oxygen changes in working skeletal muscles [16–19]. This non-invasive technique uses the differential

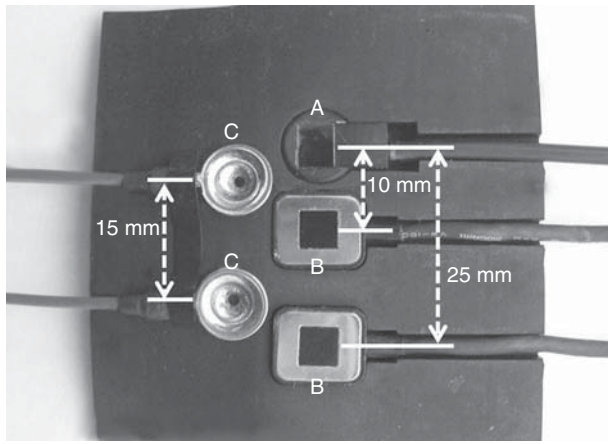


Figure 1. Placement of the light source (A) and the detectors (B) for NIRS and the EMG electrodes (C) on a rubber holder.

absorption properties of oxygenated and deoxygenated hemoglobin to evaluate blood flow and oxygen saturation in muscles [20]. As the oxygen supply in muscle is related to blood flow and changes in oxygen saturation levels are related to muscle energy metabolism, NIRS is expected to prove useful for clarifying muscle fatigue states during exercise. Several studies [21–24] have performed simultaneous recording of EMG and NIRS signals, showing that both methods reveal complementary information about muscle fatigue. However, the application of NIRS to the evaluation of muscle fatigue needs further investigation [25].

A previous study [26] investigated masseter muscle oxygenation changes and mandibular movements during experimental chewing of gum with different hardness, using NIRS and mandibular kinesiography. The results suggested that a harder texture of gum enlarges chewing motions and increases chewing velocity, with the oxygen saturation curve indicating an increase in the contribution of anaerobic metabolism to energy yield in masseter muscle. Differences in these responses to gum hardness might indicate individual differences in muscle fatigue tendencies. The purpose of this study was to clarify the relationship between changes in masseter muscle oxygenation measured by NIRS and changes in EMG spectrum during experimental chewing of gum with harder texture, to allow a better understanding of the utility of NIRS in assessing masseter muscle fatigue.

Material and methods

Subjects

Ten healthy female volunteers working at Fukuoka Dental College were examined in this study. The mean age of subjects was 28.4 ± 3.8 years. All subjects showed a Class I molar relationship and normal incisor relationships. None had any skeletal abnormalities, symptoms of temporomandibular joint dysfunction or history of chronic muscle pain in the

head or neck regions. All experimental protocols were approved by the ethics committee at Fukuoka Dental College and informed consent was obtained from all subjects prior to participation.

Experimental conditions of gum hardness

The experimental conditions of gum hardness were determined based on the results of our previous study [26]. Gum hardness was set so that, in NIRS measurement of the masseter muscle during gum chewing with a regulated chewing rhythm, the oxygen saturation level would decrease from the start of chewing and would then stabilize with a break point between these two phases. This break point would indicate changes in the pattern of oxygen consumption and energy metabolism; that is, an increase in the contribution of anaerobic metabolism to energy yield for sustained chewing [26]. The gum used in the present study was provided by Lotte Co. (Tokyo, Japan). The size of a piece of gum was $18 \text{ mm} \times 12 \text{ mm} \times 3 \text{ mm}$ (1 g). As a physical property of the gum, fracture stress after 3-min of ordinary chewing was measured using a creep meter (Model RE2-3305B; Yamaden, Tokyo, Japan) in 10 samples. Mean fracture stress was $12.5 \times 10^4 \text{ N/m}^2$.

Preparation for NIRS and EMG recordings from masseter muscle

Oxygen saturation and EMG activity during gum chewing were recorded simultaneously. Prior to measurement, the voluntary chewing side was decided by having the subject chew gum freely for 3 min and asking the subject which side was preferred. The gum was kept in a small laboratory dish.

Oxygen saturation levels were monitored continuously using a 3-wavelength NIRS laser blood oxygenation monitor (OMEGAMONITOR BOM-L1 TRW; Omegawave, Tokyo, Japan). This apparatus employs semiconductor lasers at different wavelengths (780 nm, 810 nm and 830 nm) and enables continuous, non-invasive measurement of oxyhemoglobin and deoxyhemoglobin concentrations as absolute values. The oxygen saturation level is calculated as the ratio of oxyhemoglobin in total hemoglobin (oxyhemoglobin plus deoxyhemoglobin) concentrations.

In the NIRS measurement, the light-emitting probe and two detectors were placed on a rubber holder at distances of 10 mm and 25 mm between the light source and each of the two detectors (Figure 1). Theoretical measurement ranges with each detector were a 10-mm and a 25-mm radius from the light source, respectively (Figure 2). Because oxyhemoglobin, deoxyhemoglobin and total hemoglobin concentrations within an area from 10–25 mm deep could be obtained from differences between those parameters of the two radii, oxygen saturation could

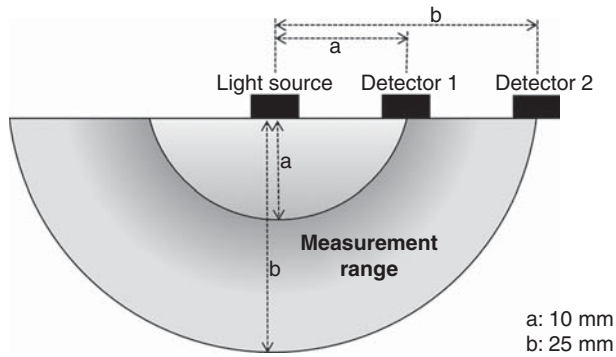


Figure 2. Distance between light source and detectors and measurement depths of NIRS. The measurement range is indicated as a darkly-shaded area.

also be calculated in the same range. For EMG recordings, bipolar surface electrodes (MLAWBT9 EEG Flat Electrodes, AD Instruments, Bella Vista, Australia) were placed on the holder bilaterally adjacent to the light-emitting probe and the detector with an inter-electrode distance of 15 mm.

After the skin of the subject was rubbed with skin-preparation gel, the measurement holder was placed in the middle of the masseter muscle antero-posteriorly on the voluntary chewing side, parallel to the main direction of muscle fibers as determined from palpation of the muscle. The holder was attached with medical double-sided tape on the masseter muscle so as to be positioned across the line connecting the tragus of the ear and the angle of the mouth (Figure 3) [26]. Prior to the experiment, absence of perturbations of NIRS and EMG data due to simultaneous recordings had been verified.

Data recordings

Each subject was placed in a dental chair with a natural head and relaxed position. Subjects were instructed to chew gum for 60 s (75 strokes) on the voluntary chewing side at a physiological pace of

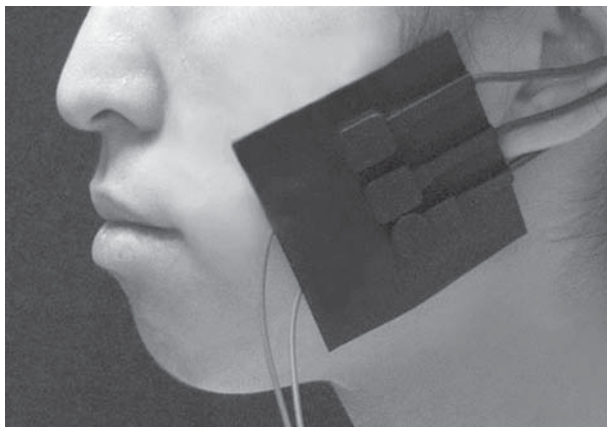


Figure 3. Simultaneous recordings of NIRS and EMG signals from masseter muscle.

1.25 strokes/s [27], in time with a metronome. Oxygen saturation levels and EMG activity were recorded from 90 s before the start of gum chewing until 150 s after the end of gum chewing.

EMG signals were amplified (ML132 Bio Amp, AD Instruments, Bella Vista, Australia) and filtered from 10 Hz to 5 kHz. The sampling frequency of the NIRS and EMG signals was 2 kHz. To normalize EMG activity during gum chewing, the activity of masseter muscle during maximum voluntary clenching was recorded for 3 s. Three measurements with a 2-min interval between them were performed and the mean of the root mean square (RMS) amplitude for each of the three repetitions was obtained.

Data analysis

EMG data processing was performed using Matlab R2011b version 7.13 software (The MathWorks, Natick, MA). The EMG indices used in this study were RMS amplitude and median frequency computed from the power spectrum in each stroke of gum chewing. RMS amplitude during gum chewing was normalized by the mean RMS amplitude during the maximum voluntary clenching described above. Because of non-stationary signals due to non-isometric contractions during gum chewing, median frequency was obtained using the wavelet transform [28–30].

Mean values at an interval of five strokes were calculated for each of three parameters: oxygen saturation level; normalized EMG amplitude; and median frequency. To evaluate changes in the three parameters during gum chewing, six intervals were set in each subject as follows.

- Interval 1: The five strokes from the sixth to the tenth stroke after starting gum chewing;
- Interval 2: The median five strokes between intervals 1 and 3;
- Interval 3: The five strokes just before appearance of the break point;
- Interval 4: The five strokes just after appearance of the break point;
- Interval 5: The median five strokes between intervals 4 and 6; and
- Interval 6: The five strokes just before the end of gum chewing.

The break point was defined as the point of intersection between two tangents drawn from the start and finish of chewing on the oxygen saturation curve [26], as shown in Figure 4.

Statistical analyses were performed using SPSS[®] Statistics version 20 (SPSS, Chicago, IL). To determine significant differences in the three parameters between the six intervals, data were subjected to one-way analysis of variance with Fisher's PLSD multiple comparison test. Values of $p < 0.05$ were regarded as statistically significant.

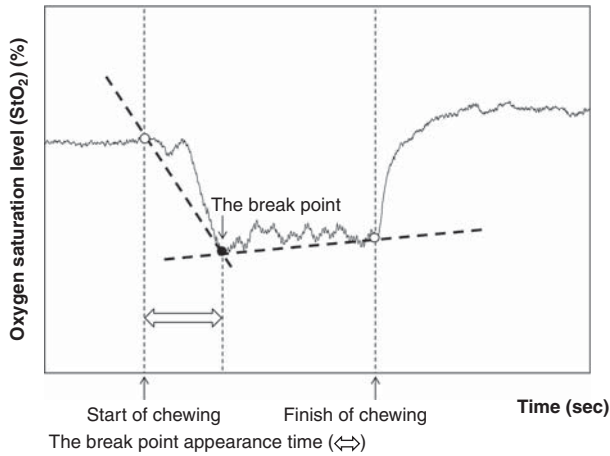


Figure 4. Definition of the break point of the oxygen saturation curve. The time of break point appearance was identified as an intersection of the two tangents from the start and finish of chewing on the oxygen saturation curve.

Results

Figure 5 shows an example of raw signal recordings of NIRS and EMG throughout the experiments. After the start of gum chewing, the oxygen saturation level decreases, then stops decreasing and stabilizes with a break point, followed by an increase after the stop of chewing. Total hemoglobin concentration increases during gum chewing. The concentration of deoxyhemoglobin increases up to the break point and then

becomes constant, while oxyhemoglobin decreases up to the break point. All subjects showed similar changes in hemoglobin concentrations and oxygen saturation levels with the break point.

Figures 6,7,8 show comparisons of oxygen saturation levels, normalized EMG amplitudes and median frequency between the six intervals, respectively.

For oxygen saturation levels, means and standard deviations (SDs) of intervals 1–6 were $61.2 \pm 4.7\%$, $57.5 \pm 6.0\%$, $52.3 \pm 8.3\%$, $50.8 \pm 9.4\%$, $51.6 \pm 8.4\%$ and $52.2 \pm 8.1\%$, respectively. The oxygen saturation level of interval 2 was significantly lower than that of interval 1 ($p < 0.05$). Oxygen saturation levels of intervals 3–6 were significantly lower than those of intervals 1 and 2 ($p < 0.01$), whereas no significant differences were seen between intervals 3–6.

For normalized EMG amplitudes, means and SDs of intervals 1–6 were $63.5 \pm 11.3\%$, $61.6 \pm 14.9\%$, $69.6 \pm 16.5\%$, $69.1 \pm 18.6\%$, $71.1 \pm 12.4\%$ and $65.2 \pm 16.9\%$, respectively. EMG amplitudes of intervals 3–5 were significantly larger than that of interval 2 ($p < 0.05$). Interval 5 also showed a larger value than interval 1 ($p < 0.05$).

Median frequency shifted lower during gum chewing. Means and SDs of median frequency for intervals 1–6 were 127.7 ± 21.1 Hz, 126.7 ± 16.3 Hz, 125.4 ± 12.1 Hz, 117.9 ± 16.1 Hz, 116.1 ± 19.2 Hz and 116.6 ± 17.3 Hz, respectively. Intervals 4–6, defined as intervals set after the break point, showed

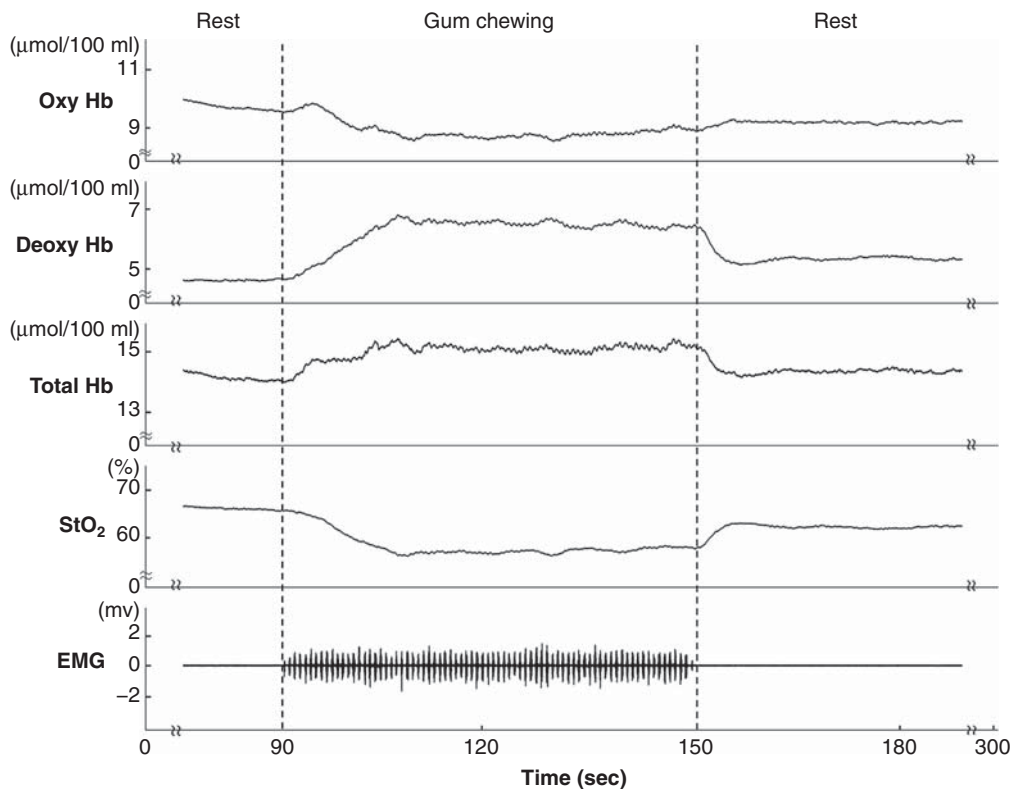


Figure 5. Representative raw recordings of NIRS and EMG signals during gum chewing. Oxy Hb, oxygenated hemoglobin; Deoxy Hb, deoxygenated hemoglobin; Total Hb, total hemoglobin; StO₂, oxygen saturation level; EMG, EMG amplitude.

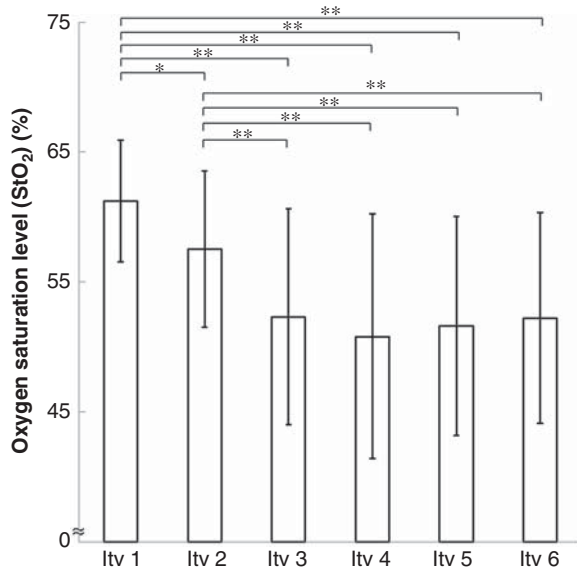


Figure 6. Changes in oxygen saturation level during gum chewing. Columns and vertical bars indicate means and standard deviations for the six intervals, respectively. * $p < 0.05$. ** $p < 0.01$. Itv 1, Interval 1; Itv 2, Interval 2; Itv 3, Interval 3; Itv 4, Interval 4; Itv 5, Interval 5; Itv 6, Interval 6.

significantly smaller values than intervals 1 and 2 ($p < 0.05$). Intervals 5 and 6 also showed significantly smaller values than interval 3 ($p < 0.05$). No significant differences were seen between intervals 1–3 or between intervals 4–6.

Discussion

In a previous study [26], we used a 2-wavelength NIRS laser blood oxygenation monitor to measure

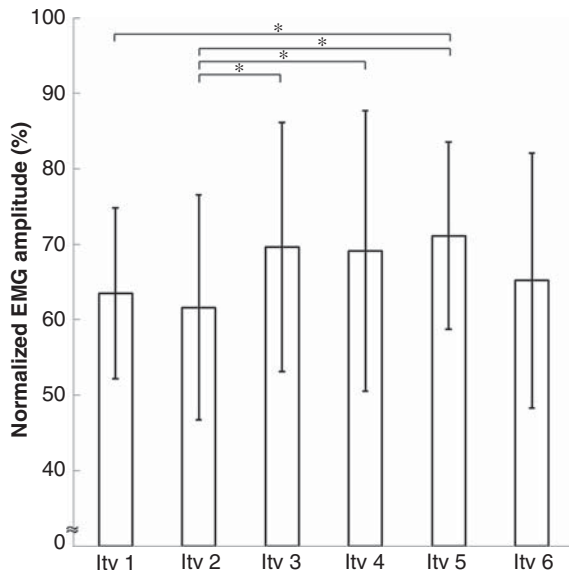


Figure 7. Changes in normalized EMG amplitude during gum chewing. Columns and vertical bars indicate means and standard deviations for the six intervals, respectively. * $p < 0.05$. Itv 1, Interval 1; Itv 2, Interval 2; Itv 3, Interval 3; Itv 4, Interval 4; Itv 5, Interval 5; Itv 6, Interval 6.

masseter muscle oxygen saturation. With the 2-wavelength apparatus, however, hemoglobin concentrations were obtained as relative values. In contrast, a 3-wavelength apparatus can measure hemoglobin concentrations as absolute values [31]. The present study used a 3-wavelength apparatus with two light detectors at different distances from the light source. This can simultaneously measure hemoglobin concentrations in two ranges from the skin surface to different depths and enables measurement of hemoglobin concentrations within a certain intermediate range by calculating differences between the two ranges. Using these methods, we have attempted to measure tissue oxygenation in the masseter muscle with the minimum influences of the subcutaneous tissue. Sugisaki et al. [32] measured thickness of the masseter muscle on magnetic resonance imaging in healthy Japanese adults and reported that the mean distances from the skin to the lateral and medial surfaces of the masseter muscle were 9.4 mm and 24.8 mm, respectively, in men and 9.8 mm and 24.0 mm in women. In reference to these results, we set measurement ranges from 10–25 mm deep to the skin surface by adjusting intervals of the light source and detectors.

Frequency analysis of EMG signals using the Fast Fourier Transform has been widely used to evaluate muscle fatigue [25]. Shifts in mean or median frequency in the power spectrum are common indicators of muscle fatigue [6–11]. However, the Fast Fourier Transform requires that the signal be stationary, thus limiting use to sustained isometric contractions. When the EMG signal is recorded during dynamic muscle contractions, the frequency content of the

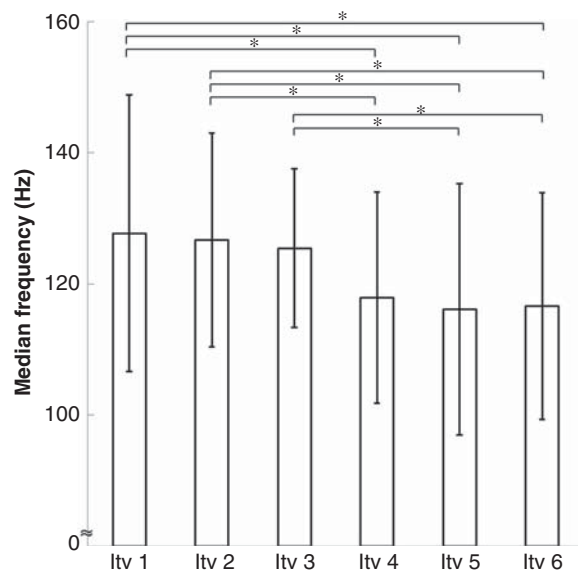


Figure 8. Changes in median frequency during gum chewing. Columns and vertical bars indicate means and standard deviations for the six intervals, respectively. * $p < 0.05$. Itv 1, Interval 1; Itv 2, Interval 2; Itv 3, Interval 3; Itv 4, Interval 4; Itv 5, Interval 5; Itv 6, Interval 6.

signal changes over time, resulting in non-stationary signals [25]. The wavelet transform has recently been developed to deal with such non-stationary signals [28], providing a more precise estimation of EMG signals under dynamic conditions [29,30]. As this study recorded masseter muscle EMG signals during dynamic contractions of gum chewing, the wavelet transform was used for frequency analysis.

Several studies [21–24] have performed simultaneous recordings of EMG and NIRS signals to clarify relationships between both signals. Yoshitake et al. [22] recorded EMG and NIRS signals simultaneously from lower-back muscles during isometric back extensions. From a comparison of changes in mean power frequency of the EMG signal, muscle blood volume and muscle oxygenation, they reported that the restriction of blood flow due to high intramuscular mechanical pressure is one of the most important factors in muscle fatigue. Praagman et al. [23] showed a high correlation between oxygen consumption rate and EMG activity on the biceps brachii and brachioradialis muscles after the start of isometric elbow flexion contractions. Yamada et al. [24] showed significant relationships between the decrease in the slope of the mean power frequency and maximal changes in oxygenated hemoglobin/myoglobin or in deoxygenated hemoglobin/myoglobin on the vastus lateralis muscle during sustained knee extension and concluded that those NIRS changes would indicate muscle fatigue assessed by EMG. Those studies suggested the possibility of detecting or predicting muscle fatigue using NIRS. The present study investigated the relationships of changes in median power frequency and oxygen saturation state on the masseter muscle during experimental gum chewing, to evaluate usefulness of NIRS for masseter muscle fatigue assessment, particularly focusing on the break point of the oxygen saturation curve.

In the present study, the oxygen saturation level decreased after the start of chewing and then stopped decreasing, showing a break point. Deoxygenated hemoglobin level increased up to the break point and then became almost constant, indicating that changes in oxygenated hemoglobin to deoxygenated hemoglobin for oxygen supply became reduced at the break point. The comparison of oxygen saturations between the six intervals seems to show that the oxygen saturation state is divisible into two phases: a decreasing phase; and a constant phase around the break point. As a pattern of force generation, known as the size principle, the brain first recruits smaller motor units that consist of slow-twitch (type 1) fibers for less force generation, then larger motor units that contain fast-twitch (types 2A and 2B) fibers are recruited and force generation increases accordingly. Type 1 and 2A fibers mostly rely on aerobic metabolism for energy, whereas type 2B fibers mostly rely on anaerobic (glycolytic) metabolism for

energy [33,34]. From the results of the previous study [26], the gum used here is hard enough that chewing force would be generated by both slow- and fast-twitch fibers under the experimental conditions. The decreasing phase of the oxygen saturation curve indicates that oxygen consumption for aerobic metabolism to energy supply proceeded rapidly. The constant phase of the oxygen saturation curve indicates that anaerobic metabolism in type 2B fibers for energy plays a major role in sustaining chewing after the break point. This anaerobic metabolism is accompanied by the accumulation of lactate and thus a decrease in intracellular pH, impeding force generation [33].

Many studies have shown that, during sustained isometric contractions, EMG signal amplitude increases and the power spectrum shifts toward lower frequencies [6–11,35]. These changes in the EMG signal are considered to be due to signal synchronization [36], modulation of the recruitment firing rate [37] and slowing of the conduction velocity [6,7,9,10]. In the present study, the RMS value increased during gum chewing, which might have been attributable to increases in motor unit recruitment and firing rate [38] while continuing to chew the harder gum. However, no clear relationships were found between break point appearance and the timing of increases in EMG amplitude.

Median power frequency decreased during gum chewing. Several studies have investigated masseter muscle fatigue during maximal or submaximal clenching and have shown the usefulness of frequency analysis for detecting masticatory muscle fatigue [12–15]. These studies showed power spectrum shifts to lower frequencies in isometric masseter muscle contractions, whereas the present study revealed those in dynamic contractions. Median frequencies for the last three intervals set after the break point were significantly lower than those for the first two intervals. No significant differences were found between the first three intervals or between the last three intervals. These results seem to indicate that the timing of break point appearance is related to the timing of a significant decrease in median frequency. Studies have reported that a reduction in conduction velocity of the action potential along the muscle fiber is the main cause of the spectral shift to lower frequencies [6,7,9,10]. The accumulation of lactic acid decreases intracellular pH, resulting in reduced excitability of the membrane and thus a decrease in action potential conduction velocity [13,33]. As described above, in the NIRS measurement, the break point of the oxygen saturation curve indicates that anaerobic metabolism plays a major role in energy supply with the accumulation of lactate [26]. The frequency shift in EMG power spectrum and the break point appearance in the oxygen saturation changes are thus considered to be attributable to the same physiological

phenomenon, namely changes in the pattern of energy metabolism. These suggest that the appearance of the break point in the NIRS measurement could be used as an indicator of masseter muscle fatigue as assessed by EMG power spectrum shifts. This would have the advantage of being easily detectable by monitoring muscle oxygen saturation. The appearance of the break point during chewing of specific boluses or the timing of its appearance from the start of chewing could be expected to be used as parameters for evaluating individual differences in masseter muscle fatigue tendencies.

Conclusions

Simultaneous recordings of EMG and NIRS signals from masseter muscle were performed during the experimental chewing of gum with harder texture. Oxygen saturation level decreased from the start of chewing, then stabilized with a break point that would indicate an increase in the contribution of anaerobic metabolism to energy yield. The results suggest that the appearance of the break point in the NIRS measurement could be used as an indicator of masseter muscle fatigue, as assessed by the EMG power spectrum shift to lower frequencies.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- [1] Farella M, Bakke M, Michelotti A, Marotta G, Martina R. Cardiovascular responses in humans to experimental chewing of gums of different consistencies. *Arch Oral Biol* 1999;44: 835–42.
- [2] Farella M, Bakke M, Michelotti A, Martina R. Effects of prolonged gum chewing on pain and fatigue in human jaw muscles. *Eur J Oral Sci* 2001;109:81–5.
- [3] Mao J, Stein RB, Osborn JW. Fatigue in human jaw muscles: a review. *J Orofac Pain* 1993;7:135–42.
- [4] Gay T, Maton B, Rendell J, Majourau A. Characteristics of muscle fatigue in patients with myofascial pain–dysfunction syndrome. *Arch Oral Biol* 1994;39:847–52.
- [5] Tortopidis D, Lyons MF, Baxendale RH. Bite force, endurance and masseter muscle fatigue in healthy edentulous subjects and those with TMD. *J Oral Rehabil* 1999;26:321–8.
- [6] Lindström L, Kadefors R, Petersén I. An electromyographic index for localized muscle fatigue. *J Appl Physiol* 1977;43: 750–4.
- [7] Viitasalo JH, Komi PV. Signal characteristics of EMG during fatigue. *Eur J Appl Physiol Occup Physiol* 1977;37:111–21.
- [8] Petrofsky JS, Glaser RM, Phillips CA, Lind AR, Williams C. Evaluation of the amplitude and frequency components of the surface EMG as an index of muscle fatigue. *Ergonomics* 1982; 25:213–23.
- [9] Eberstein A, Beattie B. Simultaneous measurement of muscle conduction velocity and EMG power spectrum changes during fatigue. *Muscle Nerve* 1985;8:768–73.
- [10] Arendt-Nielsen L, Mills KR, Forster A. Changes in muscle fiber conduction velocity, mean power frequency, and mean EMG voltage during prolonged submaximal contractions. *Muscle Nerve* 1989;12:493–7.
- [11] Masuda K, Masuda T, Sadoyama T, Inaki M, Katsuta S. Changes in surface EMG parameters during static and dynamic fatiguing contractions. *J Electromyogr Kinesiol* 1999;9:39–46.
- [12] Palla S, Ash MM Jr. Power spectral analysis of the surface electromyogram of human jaw muscles during fatigue. *Arch Oral Biol* 1981;26:547–53.
- [13] Lindström L, Hellsing G. Masseter muscle fatigue in man objectively quantified by analysis of myoelectric signals. *Arch Oral Biol* 1983;28:297–301.
- [14] Lyons MF, Rouse ME, Baxendale RH. Fatigue and EMG changes in the masseter and temporalis muscles during sustained contractions. *J Oral Rehabil* 1993;20:321–31.
- [15] Castrorflorio T, Falla D, Tartaglia GM, Sforza C, Deregibus A. Myoelectric manifestations of jaw elevator muscle fatigue and recovery in healthy and TMD subjects. *J Oral Rehabil* 2012;39:648–58.
- [16] Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near-infrared spectroscopy in humans. *J Appl Physiol* 1994;77:2740–7.
- [17] Belardinelli R, Barstow TJ, Porszasz J, Wasserman K. Changes in skeletal muscle oxygenation during incremental exercise measured with near infrared spectroscopy. *Eur J Appl Physiol Occup Physiol* 1995;70:487–92.
- [18] Bhambhani Y, Maikala R, Esmail S. Oxygenation trends in vastus lateralis muscle during incremental and intense anaerobic cycle exercise in young men and women. *Eur J Appl Physiol* 2001;84:547–56.
- [19] Fadel PJ, Keller DM, Watanabe H, Raven PB, Thomas GD. Noninvasive assessment of sympathetic vasoconstriction in human and rodent skeletal muscle using near-infrared spectroscopy and Doppler ultrasound. *J Appl Physiol* 2004;96: 1323–30.
- [20] Chance B, Cope M, Gratton E, Ramanujim N, Tromberg B. Phase measurement of light absorption and scatter in human tissue. *Rev Sci Instrum* 1998;69:3457–81.
- [21] Miura H, Araki H, Matoba H, Kitagawa K. Relationship among oxygenation, myoelectric activity, and lactic acid accumulation in vastus lateralis muscle during exercise with constant work rate. *Int J Sports Med* 2000;21:180–4.
- [22] Yoshitake Y, Ue H, Miyazaki M, Moritani T. Assessment of lower-back muscle fatigue using electromyography, mechanomyography, and near-infrared spectroscopy. *Eur J Appl Physiol* 2001;84:174–9.
- [23] Praagman M, Veeger HE, Chadwick EK, Colier WN, van der Helm FC. Muscle oxygen consumption, determined by NIRS, in relation to external force and EMG. *J Biomech* 2003;36:905–12.
- [24] Yamada E, Kusaka T, Arima N, Isobe K, Yamamoto T, Itoh S. Relationship between muscle oxygenation and electromyography activity during sustained isometric contraction. *Clin Physiol Funct Imaging* 2008;28:216–21.
- [25] Al-Mulla MR, Sepulveda F, Colley M. A review of non-invasive techniques to detect and predict localised muscle fatigue. *Sensors* 2011;11:3545–94.
- [26] Yoshida T, Ishikawa H, Yoshida N, Hisanaga Y. Analysis of masseter muscle oxygenation and mandibular movement during experimental gum chewing with different hardness. *Acta Odontol Scand* 2009;67:113–21.
- [27] Lavelle CL. *Applied oral physiology*. 2nd ed. London: Wright; 1988. p 12–24.
- [28] Samar VJ, Bopardikar A, Rao R, Swartz K. Wavelet analysis of neuroelectric waveforms: a conceptual tutorial. *Brain Lang* 1999;66:7–60.
- [29] Karlsson S, Yu J, Akay M. Time-frequency analysis of myoelectric signals during dynamic contractions: a comparative study. *IEEE Trans Biomed Eng* 2000;47:228–38.

- [30] González-Izal M, Rodríguez-Carreño I, Malanda A, Mallor-Giménez F, Navarro-Amézqueta I, Gorostiaga EM, et al. sEMG wavelet-based indices predicts muscle power loss during dynamic contractions. *J Electromyogr Kinesiol* 2010; 20:1097–106.
- [31] Narita N, Tominaga T, Kosu K, Mizoi K, Yoshimoto T. Monitoring of brain tissue haemoglobin concentration and oxygen saturation using a three wavelength spectrophotometric method. *Neurol Res* 1994;16:428–32.
- [32] Sugisaki M, Misawa A, Ikai A, Young-Sung K, Tanabe H. Sex differences in the hemoglobin oxygenation state of the resting healthy human masseter muscle. *J Orofac Pain* 2001;15:320–8.
- [33] Boron WF, Boulpaep EL. *Medical physiology. A cellular and molecular approach*. Philadelphia, PA: Saunders; 2003. p 250–3; p 1242–7.
- [34] Pocock G, Richards CD, Daly MB. *Human physiology. The basis of medicine*. 2nd ed. New York: Oxford University Press; 2004. p 102–7; p 579–83.
- [35] Hagberg M. Work load and fatigue in repetitive arm elevations. *Ergonomics* 1981;24:543–55.
- [36] Kleine BU, Stegeman DF, Mund D, Anders C. Influence of motoneuron firing synchronization on SEMG characteristics in dependence of electrode position. *J Appl Physiol* 2001;91: 1588–99.
- [37] Gazzoni M, Farina D, Merletti R. Motor unit recruitment during constant low force and long duration muscle contractions investigated with surface electromyography. *Acta Physiol Pharmacol Bulg* 2001;26:67–71.
- [38] Lippold OC, Redfearn JW, Vuco J. The electromyography of fatigue. *Ergonomics* 1960;3:121–31.