Sex Differences in the Hemoglobin Oxygenation State of the Resting Healthy Human Masseter Muscle

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Aims: *To determine whether sex differences exist in tissue oxygen saturation (StO_2) and the hemoglobin (Hb) oxygenation state of the resting human masseter muscle.* **Methods:** *Near-infrared spectroscopy (NIRS) was used to measure StO_2 and Hb oxygenation state in 20 healthy adult volunteers (10 women and 10 men). To determine the measurement range and reliability of the NIRS recording probe, the probe was set up on 12 layers of white acrylic resin plate, each 3 mm thick. Total hemoglobin levels were measured while a red vinyl resin plate, 1 mm thick, was inserted in turn between each of the 12 layers. Distances from the skin surface to the lateral surface (S-L) and to the medial surface (S-M) of the right masseter at the middle portion of the masseter were measured on T1-weighted magnetic resonance images (repetition time 500 ms, echo time 23 ms). Thickness of the masseter was calculated by subtraction [(S-M) – (S-L)]. For the study of Hb oxygenation state, the probe was positioned at the same position on the skin surface at the mandibular postural (rest) position.* **Results:** *The measurement range of the NIRS probe was from 9 to 21 mm under the skin, and the reliability of the probe was judged by intra- and inter-class correlation coefficients. There was no sex difference in S-L and the thickness of the masseter; the means of S-L and masseter thickness were 9.3 mm and 15.5 mm in men and 9.8 mm and 14.3 mm in women, respectively. Except for StO_2 values, there were significant sex differences in the Hb oxygenation parameters, with the mean values in the men being approximately twice those in the women.* **Conclusion:** *These results provide evidence that a sex difference in the Hb oxygenation state may exist in the masseter muscle of normal healthy subjects.*

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Key words: hemoglobin oxygenation, hemodynamics, blood flow, masseter muscle

Several biologic studies have indicated that there may be sex differences in the shapes and functions of human skeletal muscles.[1,2] The body compositions of adult men and women differ in that women have a greater proportion of body fat and a lower lean body mass. Lower hematocrit and hemoglobin (Hb) content values have also been shown in women.[3] Basal cutaneous blood flow is also lower in women,[4] but there is no sex difference in the percentage arterial oxygen saturation of Hb (SaO_2), which is about 97.5% in both sexes.[5] In the masticatory muscles, sex differences in the jaw-jerk reflex have been reported, with women exhibiting a significantly shorter mean latency but a significantly higher amplitude than men.[6] Blood flow in the masticatory muscles could be an important factor in determining such functional differences between the sexes. However, there have been no reports regarding sex differences in the Hb oxygenation state of the masseter muscle.

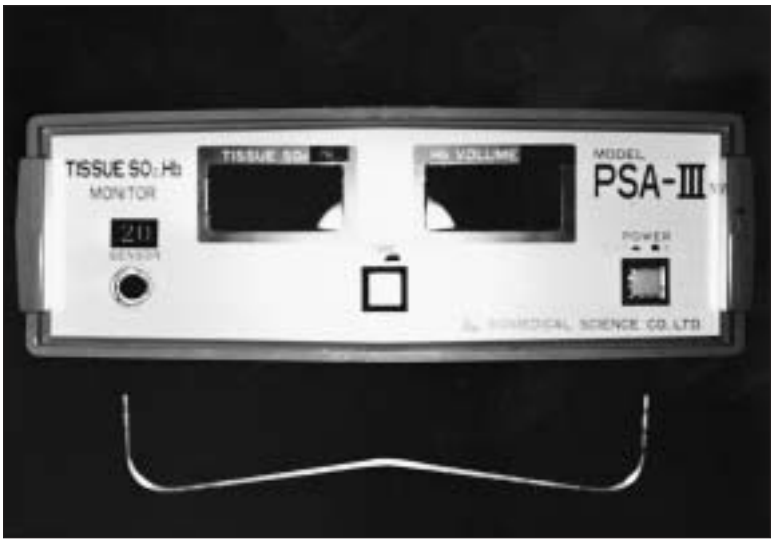

Fig 1 The NIRS StO_2/Hb monitor (PSA-IIIN; Biomedical Science).

Several noninvasive techniques have recently been developed for the evaluation of muscle blood flow, including color Doppler imaging, magnetic resonance imaging (MRI)/angiography, and near-infrared spectroscopy (NIRS). The latter is a relatively inexpensive method that uses a portable probe to measure changes in tissue oxygen saturation (StO_2) and can provide information on oxygen delivery to muscle tissue. Near-infrared spectroscopic monitoring of StO_2 and/or the Hb oxygenation state may therefore provide valuable information on circulation and metabolism within the masseter muscle. The purpose of this study was to use NIRS to examine whether sex differences exist in the StO_2 and Hb oxygenation state of the resting normal human masseter muscle.

Materials and Methods

Apparatus

The StO_2 and the Hb oxygenation state of the masseter muscle were measured with the mandible in the postural (rest) position[7] by a 3-wavelength NIRS StO_2/Hb monitor (PSA-IIIN, Biomedical Science) (Fig 1). This apparatus enables continuous, noninvasive measurement of the Hb oxygenation state. The rectangular recording probe (2×5 cm) comprises a set of 3 light-emitting diodes (LEDs), each of which emits light of a different wavelength (700, 750, and 830 nm), and 2 light intensity detectors. The distance between the LEDs and detector A is 10 mm and that between detectors A and B is 15 mm (Fig 2). The theoretical measurement range is within a 10- to 25-mm radius of the light source when the monitor is placed on the skin surface (Fig 3). The apparatus has 4 output-monitoring channels measuring oxygenated, deoxygenated, and total Hb (OXHb, deOXHb, and THb) and StO_2, and these were used as appropriate for each part of the study. Oxygenated Hb and deOXHb levels were measured directly and expressed as absolute values according to Beer-Lambert's law and simultaneous equations.[8] Total Hb levels (THb = OXHb + deOXHb) and StO_2 levels (StO_2 = OXHb/THb) were also calculated.

Participants

Volunteers were recruited from the staff at Tsurumi University, Tsurumi City, Japan. Informed consent was obtained from all volunteers before they entered the study. They then gave a verbal medical history, and their personal health records were checked to determine any current or previous medical history of anemia, cardiovascular disease, or other clinical conditions. Blood tests for hematocrit (%) and Hb content (g/dL) were performed. Percentage arterial oxygen saturation of Hb (%) and pulse rate were monitored during the measurements. The participants were also checked for masticatory abnormalities, and any primary diagnosis of temporomandibular disorders (TMD) was made in accordance with the criteria of Fricton et al.[9] Subjects were excluded from the study if the blood tests showed values outside the

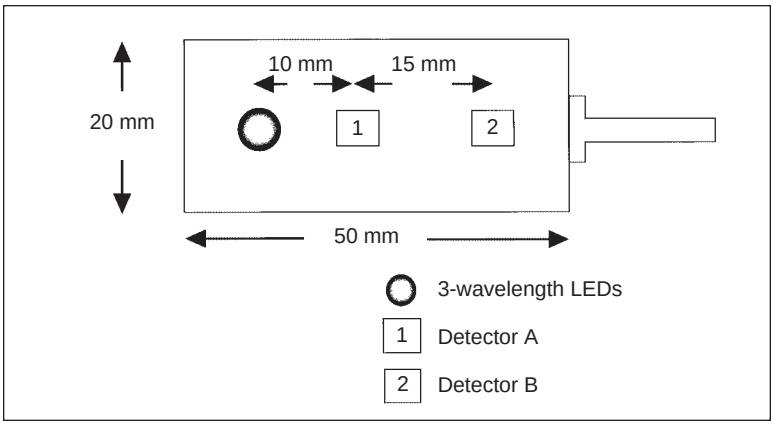

Fig 2 Diagram of recording probe of the NIRS (PSA-IIIN).

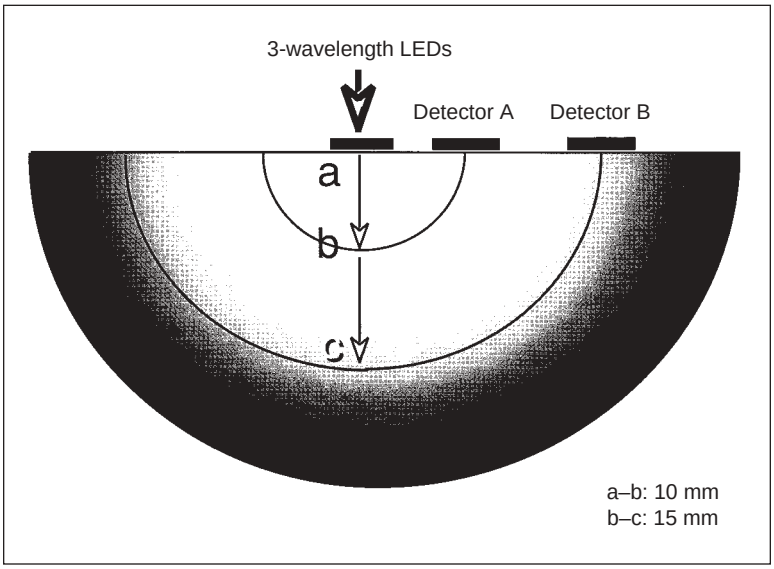

Fig 3 Theoretical measurement range of the NIRS (PSA-IIIN).

normal ranges, if they had anemia or cardiovascular disease (or a history of such conditions), or if they had primary TMD. Twenty healthy volunteers (aged 23 to 45 years, 50% women) were included in the study (Table 1).

Study Design

This study included 4 investigations: confirmation of the measurement range of the PSA-IIIN, confirmation of the reliability of the PSA-IIIN, determination of the depth and thickness of the masseter muscle in the healthy volunteers by MRI, and measurement of the Hb oxygenation state of the masseter muscle in the same volunteers. The protocol for each experiment is summarized in Table 2.

Confirmation of the Measurement Range and Reliability of the PSA-IIIN. As stated above, the theoretical measurement range of the monitor is within a 10- to 25-mm radius of the light source when it is placed on the skin surface, but the actual measurement range and reliability of the PSA-IIIN were unknown. The probe was therefore set up on 12 layers of white acrylic resin plate, each 3 mm thick (total thickness 36 mm), and 3 kg of pressure was applied to the probe. Total Hb was measured while a 1-mm-thick red vinyl resin plate was inserted between each of the white acrylic resin layers in turn, from the bottom upward. As a control, THb was also measured without insertion of the red vinyl resin plate.

The measurements were performed over 60 seconds with a sampling frequency of 1 Hz and were repeated 4 times to determine the measurement range of the apparatus. For the measurement reliability study, the measurements were performed 3

Table 1 Subject Data (Mean and 95% Confidence Intervals)

	Men (n = 10)	Women (n = 10)	95% CI between sexes
Mean age (y)	30.5 (26.9–34.1)	27.1 (24.4–29.8)	–7.56 to 0.76
Mean body weight (kg)	68.6 (60.7–76.5)	50.8 (45.8–55.8)	–26.47 to –9.12
Mean height (cm)	173.9 (169.1–178.7)	158.7 (155.3–162.1)	–20.67 to –9.73
Oxygen saturation (%)	97.4 (96.4–98.4)	97.7 (97.3–98.2)	–0.70 to 1.38
Hematocrit (%)	47.3 (45.0–49.7)	41.8 (40.1–43.5)	–8.26 to –2.87
Hemoglobin content (g/dL)	15.5 (14.7–16.3)	13.7 (13.1–14.3)	–2.75 to –0.90

Table 2 Experimental Protocols

Experiment 1 Measurement range of PSA-IIIN	Experiment 2 Measurement reliability of PSA-IIIN	Experiment 3 Depth and thickness of the masseter muscle by MRI	Experiment 4 Masseter muscle hemoglobin oxygenation state
Monitoring channel: THb Measurement time: Over 60 seconds Sampling frequency: 1 Hz Calculation: Summed up the data of the middle 10 seconds Measurement times: Four times on the same day	Monitoring channel: THb Measurement time: Over 60 seconds Sampling frequency: 1 Hz Calculation: Summed up the data of the middle 41 seconds Measurement times: Three times on a different day	Subjects: 20 volunteers (50% women), mean age: men 30.5 years and women 27.1 years Apparatus: Hitachi MRP7000 (0.5T) with field of view = 240 mm/256, T1-weighted Measurement site: Cervical plane of the maxillary molar teeth level	Subjects: The same 20 volunteers (50% women) Apparatus: PSA-IIIN Measurement site: Cervical level of the maxillary molar teeth Monitoring channel: THb, deOXHb, OXHb, and StO_2 Measurement time: Over 80 seconds Sampling frequency: 1 Hz Calculation: Summed up the data of the middle 60 seconds

times on different days. The first and several of the final measurements were rejected, and those for the middle 10 and 41 seconds were used for the measurement range and reliability tests, respectively.

Determination of the Depth and Thickness of the Masseter Muscle by MRI. We were unable to locate any published information on the depth and thickness of the right side of the masseter muscle at the level of the cervical region of the maxillary molars, where the PNS-IIIN probe was to be placed for the Hb oxygenation state study. Therefore, T1-weighted (repetition time 500 ms, echo time 23 ms) MRI axial images of the masseter muscle were obtained with a Hitachi MRP 7000 (0.5 T; field of view = 240 mm/256), and the distances from the skin surface to the lateral (S-L) and medial (S-M) surfaces of the middle portion of the muscle were measured at the cervical level of the maxillary molars. The thickness of the masseter muscle was calculated by subtracting S-L from S-M.

Measurement of the Masseter Muscle Hemoglobin Oxygenation State. The same 20 healthy volunteers underwent this part of the study just after the MRI procedure. The PSA-IIIN probe was positioned on the right side of the face above the masseter muscle, at the level of the maxillary molars, and was secured to the skin surface with double-sided adhesive tape. Each subject was seated upright in a comfortable position in a dental chair and instructed to relax his or her jaw for 5 minutes. The 4 channels were then set to record continuously over 80 seconds and the data were sampled at a frequency of 1 Hz. Because we wished to obtain a value for a measurement time of precisely 60 seconds, the first and several of the final measurements were rejected, and those for the middle 60 seconds were added together for each channel.

Statistical Analysis

When the data were normally distributed according to the Lilliefors and Shapiro-Wilks test,[10] they were expressed as mean values. The 95% confidence intervals (95% CI) for the differences between the sexes were analyzed by an independent *t* test with the Levene test.[10] For multiple-comparison tests of the actual measurement range of the PSA-IIIN, statistical analyses were conducted on parametric data because of the robust-

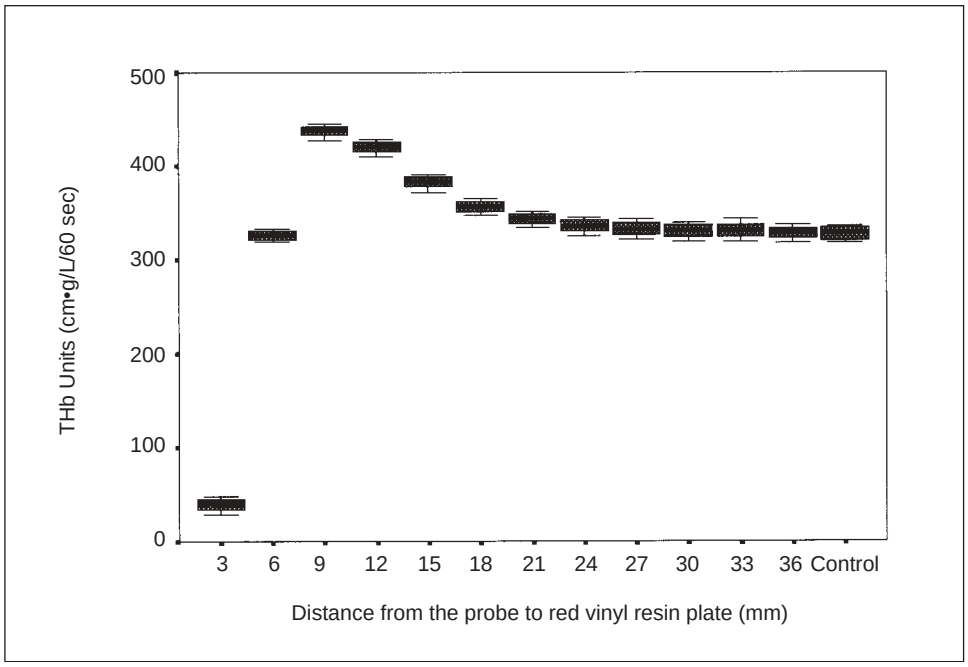

Fig 4 Box-whisker plot showing the experimental total hemoglobin (THb) measurements for each white resin layer in terms of distance from probe to a red vinyl resin plate placed in turn between each white resin layer. Control was measured without insertion of the red vinyl resing plate.

ness of the 1-way layout analysis of variance with the Levene test (1-way analysis of variance [ANOVA] with the Tukey honestly significant difference [HSD] test). A correlation coefficient value was calculated by the Pearson correlation. All analyses were performed with SPSS software (Release 8j, Tokyo). A probability level of less than .05 (2-tailed) was regarded as significant. The reliability of the PSA-IIIN was analyzed by intra- and interclass correlation coefficients (ICC)[11] obtained from a 2-way ANOVA model.[12,13] The range of ICC values defining each level of class agreement were as follows: 0.00 to 0.39 = unacceptable, 0.40 to 0.59 = moderate, 0.60 to 0.79 = good, and 0.80 to 1.00 = excellent.

Results

There were no statistically significant differences between the sexes with respect to age and SaO_2. There were, however, significant differences between the sexes with respect to Hb content, hematocrit, body weight, and height, although all values were within the normal range (Table 1). The differences between the sexes for height and weight were 15.2 cm and 17.8 kg, respectively; the correlation coefficient value between height and masseter thickness was 0.882 ($P < .01$), and that between weight and masseter thickness was 0.537 ($P < .05$).

Confirmation of the Measurement Range and Reliability of the PSA-IIIN

Box-whisker plots of the THb measurements for each resin layer are shown in Fig 4. In the 10- to 25-mm range, ie, within the theoretical measurement range of the apparatus, the Tukey HSD test revealed that the values for layer thicknesses of 9, 12, 15, 18, and 21 mm were significantly higher than the control values ($P < .05$). The values for the 9- and 12-mm-thick layers were 1.33 and 1.28 times higher, respectively, than the control values, while those for the 18- and 21-mm-thick layers were 1.08 and 1.04 times higher, respectively. There were no other significant differences between the measured and control values, except for the 3-mm-thick layer.

The reliability of the monitor was documented by ICC analyses. The ICCs ranged from 0.97 to 0.99 and thereby demonstrated the excellent reliability of the monitor, except for the measurements on the 3-mm-thick layer (Table 3).

Demonstration of the Depth and Thickness of the Masseter Muscle by MRI

Table 4 shows the results of this experiment. The mean S-L and S-M distances were 9.4 mm (95% CI, 7.8 to 11.0) and 24.8 mm (95% CI, 21.5 to 28.1), respectively, in men, and 9.8 mm (95% CI, 9.0 to 10.5) and 24.0 mm (95% CI, 22.5 to 25.7), respectively, in women. The masseter muscle thickness was 15.5 mm (95% CI, 13.3 to 17.6) for men and 14.3 mm (95% CI, 12.8 to 15.2) for women. There were no significant differences between the sexes in these measurements.

Table 3 Reliability of PSA-IIIN

Acrylic resin thickness	Intraclass correlation coefficient
3 mm	0.0742
6 mm	0.9995
9 mm	0.9972
12 mm	0.9927
15 mm	0.9921
18 mm	0.9890
21 mm	0.9735
24 mm	0.9790
27 mm	0.9894
30 mm	0.9820
33 mm	0.9960
36 mm	0.9331
Control	0.9703

Measurement of the Hemoglobin Oxygenation State in the Resting Masseter Muscle

The Shapiro-Wilks and Lilliefors tests showed that THb, OXHb, and deOXHb did not follow a normal distribution, but StO_2 did follow a normal distribution. An independent *t* test with the Levene test was selected for the analysis because of its robustness. The 4 sets of measurements are shown in Table 5. All the data were summed to produce a mean value over 60 seconds. The StO_2 (%/60 seconds) values were 4,554 (95% CI, 4,455.5 to 4,652.5) in men and 4,578 (95% CI, 4,474.7 to 4,681.9) in women, yielding StO_2 (%/second) values of 75.9% in men and 76.3% in women. There were significant differences between the sexes for all the measurements except for StO_2, with the mean values in the men approximately double those in the women.

Discussion

In a previous study, NIRS with dual-wavelength LEDs and a single spectroscopic detector with a theoretical sampling range of 0 to 30 mm beneath the skin surface was used to measure hemodynamic changes in the female human masseter muscle.[14] However, other structures are present between the skin and the masseter muscle, and these may have affected the hemodynamic values;

Table 4 Sex Differences (Mean and 95% CI) in Depth and Thickness of the Masseter Muscles

	Men	Women	95% CI between sexes
S-L (mm)	9.4 (7.8–11.0)	9.8 (9.0–10.5)	–1.23 to 2.04
S-M (mm)	24.8 (21.5–28.1)	24.0 (22.5–25.7)	–4.22 to 2.63
Thickness (mm)	15.5 (13.3–17.6)	14.3 (12.8–15.2)	–4.31 to 2.72

S-L = distance from the skin to the lateral surfaces of the masseter muscle; S-M = distance from the skin to the medial surface of the masseter muscle; CI = confidence interval.

Table 5 Sex Differences in Hemoglobin Oxygenation State (Mean and 95% CI)

	Men (n = 10)	Women (n = 10)	95% CI between sexes
DeOXHb	6,981.4 (4,714.7–9,248.2)	3,324.6 (2,174.0–4,455.4)	–6,009.34 to –1,304.18
OXHb	21,761.6 (15,251.6–28,271.8)	10,521.3 (7,361.2–13,681.4)	–17,961.12 to –4,519.64
StO_2	4,554.01 (4,455.5–4,652.5)	4,578.3 (4,474.7–4,681.9)	–108.48 to 157.13
THb	28,743.2 (20,018.9–37,467.4)	13,846.0 (9,579.1–18,112.9)	–23,916.68 to –5,877.61

All data show the mean of the sum total values for 60 seconds of individual volunteer. Then StO_2 (%/second) were about 75.9% in men and 76.3% in women. Hb units: cm × g/1/60 second.

also, the actual measurement range of the apparatus was not demonstrated. Nevertheless, NIRS measurements with a dual-wavelength and single-detector spectrometer have been shown to be only minimally affected by skin blood flow.[15] With the dual-wavelength method, absolute values for Hb volume cannot be calculated and StO_2 values are calculated through the use of the absorption ratio of the 2 light wavelengths, which are set so that one is the iso-absorption point of Hb and deOXHb and the other is the point at which the absorption becomes noticeably different due to the presence of the 2 forms of Hb. With the dual-wavelength method, there is a possibility that changes in the Hb content of the brain tissue might conceivably introduce error.[16] In contrast, with the 3-wavelength spectrophotometric method, Hb concentrations are theoretically obtainable and a more stable recording can be expected. Furthermore, simultaneous evaluation of SaO_2 and Hb concentrations becomes possible.[8]

Measurement Range and Reliability of the PSA-IIIN

The validity of NIRS in human skeletal muscle has been reported.[15] The path length of the NIRS LED in muscle is unknown for pulsed light, although a report by Chance et al suggests that the average path length of continuous light from tungsten filament lamps in human skeletal muscle is approximately 2.6 cm.[17]

The Hb oxygenation state values in this study were measured by a 3-wavelength PSA-IIIN. When the PSA-IIIN measurements were repeated 4 times during the confirmatory experiments, the ICCs ranged from 0.97 to 0.99, thus demonstrating excellent reliability (Table 4). The actual THb measurement range of the apparatus was minimally affected at the 3-mm layer thickness; the value for this thickness was significantly lower than that of the control (about one-third). These results show that the actual measurement range of the monitor, 9 to 21 mm from the LEDs, is almost identical to the theoretical range (10 to 25 mm). The Tukey HSD test revealed that the values for layer thicknesses of 9, 12, 15, 18, and 21 mm were significantly higher than values for the control ($P < .05$); the values at layer thicknesses of 9 and 12 mm were higher than those obtained with layer thicknesses of 18 and 21 mm (Fig 4).

In this study, the men were found to have a thicker masseter muscle (15.5 mm) than the women (14.3 mm), but this difference was not significant (95% CI, –1.23 to 2.04). One previous study also reported that the human male has a thicker masseter muscle than the female.[18] The investigators used ultrasonography to determine mean values for masseter muscle thickness in postpubertal (15 to 20 years) females and males and found a significant difference in the masseter thicknesses (10.7 mm and 13.1 mm, respectively). They also stated that the masseter muscle thickness was correlated with body weight and height. The disagreement between their results and ours may be the result of differences in body build between the 2 groups of subjects. In the previous study, the differences between the sexes were 18 cm in height and 20.6 kg in weight. In our subjects, the between-sex differences were 15.2 cm in height and 17.8 kg in weight, and the correlation coefficient values (0.882 and 0.537) showed significant correlations between height and masseter thickness and between weight and masseter thickness, respectively. Therefore, if the between-sex differences in body build had been larger in our subjects, there might have been a significant difference in the masseter thickness. Thus, the possibility that the masseter muscle might be bigger in men than in women cannot be ruled out, and body weight and height were indeed significantly larger among the men. Nevertheless, the MRI results showed that the muscle usually lies at a depth of 9 to 12 mm under the skin (a depth well within the accurate measuring capability of the PSA-IIIN). The mean S-L distance was 9.4 mm in men and 9.8 mm in women, and only 1 male volunteer had a measurement outside this range (S-L = 14.3 mm). Furthermore, the 95% CIs for S-M ranged from 21.5 to 28.1 mm in men and from 22.5 to 25.7 mm in women, and the small areas that theoretically lay outside the measurable range would not be likely to have much influence on the measurement values. These results suggest that the thickness of the subcutaneous tissue and of the masseter itself had little influence on the values measured by the PSA-IIIN during this study.

Sex Differences in the Hemoglobin Oxygenation State

In spectroscopic measurements, the blood volume and Hb content, as well as the density of the capillary bed lying within the fixed measurement range, may affect OXHb, deOXHb, and THb level measurements. However, the Hb content does not affect the StO_2 level in the measurement field as a result, although ventilation, local metabolism, and capillary bed density in the mandibular rest position could affect StO_2. Although our study found

no significant differences in StO_2 between the sexes, there were significant differences in all Hb oxygenation state values, with the mean values for the men being much greater than those for the women. Unfortunately, we cannot compare these differences with those found in previous studies, because there do not appear to be any reports detailing between-sex differences for the resting masseter muscle. However, there are a few reports of sex differences in blood flow through other skeletal muscles. Jensen et al showed that gender-related differences in blood flow in the resting leg led to a smaller difference between arteriovenous O_2 and CO_2 levels in women compared with men.[19] A further study demonstrated that peak reactive hyperemic flow in the forearm was significantly greater in men than in women.[20] These reports suggest that men might have a higher density of capillaries in their skeletal muscles than women. Stal et al proposed that the masseter muscle contains a higher density of capillaries than limb muscle, and suggested that differences in the tasks performed and functional activity between these muscles were reflected by a relatively higher need and demand for blood supply in the masseter than in the limb muscles.[21]

Histochemical studies have indicated that type I muscle fibers are surrounded by a rich supply of capillaries, but type IIB fibers have fewer capillary beds.[22] There have been some reports of sex differences in the types of fibers found in the masseter muscle in primates, but these findings are controversial. In the adult rhesus monkey, the anterior section of the superficial masseter muscle shows the most significant difference between males and females in terms of the proportion of different types of muscle fiber and the sizes of these fibers. The female has markedly smaller type II muscle fibers and a greater percentage of type I fibers than the male.[23] In contrast, no significant sex differences were apparent in the central section of the superficial masseter. In addition, while female primates have a similar percentage of type I and II fibers, both of these fiber types have significantly smaller cross-sectional areas than those in males.[24] These results suggest that the type and size of the muscle fibers in the masseter may differ between the sexes, and that this might influence the Hb oxygenation state; however, the differences in masticatory function between non-human primates and humans must be borne in mind.

In the present study, the OXHb, deOXHb, and THb levels in men were about twice those seen in women, while the Hb content in men was approximately 1.1 times higher than that in women. There were no significant correlations between the Hb content and masseter thickness on the one hand and each of the Hb oxygenation level parameters on the other. Tissue oxygen saturation was about 75%/second in both men and women, with no significant difference. Furthermore, the Hb content did not affect the StO_2 level in the field of interest. These findings suggest that the higher OXHb, deOXHb, and THb levels seen in the men might be dependent on the higher capillary bed density, which may have developed originally as a physiologic sex difference.

Three-wavelength NIRS was shown to be an effective method of examining the Hb oxygenation state in the masseter muscle of adult humans. Our findings suggest that there may be a normal physiologic difference in the Hb oxygenation state between the sexes, and these should be taken into account when the Hb oxygenation state of the masseter muscle is examined. Further research will be necessary to characterize more accurately the sex differences in the Hb oxygenation state of the resting masseter muscle.

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