Hemodynamic monitoring in the human temporalis muscle using near-infrared spectroscopy

HEMODYNAMICMONITORING IN THE TEMPORALIS MUSCLE USING NEAR-INFRARED SPECTROSCOPY

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Abstract

Objective. Altered temporal muscle perfusion is implicated in several painful disorders afflicting orofacial and head regions, including temporomandibular joint dysfunctions, bruxism, and headache. Knowledge about the regulation of blood supply to the temporalis muscle is limited, due to methodological difficulties. The aim of the study was to test the feasibility of near-infrared spectroscopy (NIRS) monitoring of the human temporal muscle. *Approach*. Twenty-four healthy subjects were monitored with a 2-channel NIRS: a *muscle* probe placed over the temporal muscle and a *brain* probe placed on the forehead. A series of teeth clenching at 25, 50, and 75 % of maximum voluntary contraction for 20 sec and hyperventilation for 90 sec at 20 mmHg of end-tidal CO_2 were performed, to elicit hemodynamic changes in muscle and brain, respectively. *Main results*. Responses of NIRS signals from both probes were consistently different during both tasks. The absolute change in tissue oxygenation index (ΔTOI) as detected by muscle and brain probes was -7.80 ± 11.76 and 0.29 ± 1.42 % during teeth clenching ($p < 0.01$) at 50 % maximum voluntary contraction, while -0.92 ± 2.50 and -5.08 ± 3.78 % during hyperventilation ($p < 0.01$), respectively. *Significance*. Distinct response patterns were observed from the temporal muscle and prefrontal cortex which proves that this technique is adequate to monitor tissue oxygenation and hemodynamic changes in human temporal muscle. Noninvasive and reliable monitoring of hemodynamics in this muscle will help to extend basic and clinical investigations about the peculiar control of blood flow in head muscles.

Keywords Hemodynamics, NIRS, Human Temporal Muscle

Introduction

The human temporalis is a fan-shaped thin muscle that extends superficially from the temporal bone to the coronoid process of the mandible and serves as one of the essential masticatory muscles to perform elevation and retraction of the mandible (Yu *et al* 2021). The temporalis muscle is implicated in several painful disorders afflicting the orofacial and head regions, including temporomandibular joint dysfunctions, bruxism (Lavigne *et al* 2008, Yap and Chua 2016), and headache (Exposto *et al* 2021). Vascular dysfunctions in masticatory muscles have often been considered a possible cause of pain symptoms, related either to decreased muscle perfusion (Maekawa *et al* 2002, Shah *et al* 2019) or to excessive dilatory phenomena (Jensen 1993), although this latter issue is still debated (Jacobs and Dussor 2016, Mason and Russo 2018). It has also been suggested that blood flow is differently controlled in head muscles, compared to limb muscles but investigations in the head region are mostly limited to the masseter muscle (Nakamura *et al* 2005, Rashid and Roatta 2022). In fact, investigations about the regulation of blood flow in the temporalis muscle are scanty. The blood supply to the temporalis muscle is provided by the anterior and posterior deep temporal arteries, which are branches of the internal maxillary artery, and anastomose within the muscle with the middle temporal artery, which is a branch of the superficial temporal artery (Elazab and Abdel-Hameed 2006). For this reason, Doppler ultrasound of the superficial temporal artery, which is taken downstream to the branching of the middle temporal artery (Arbeille *et al* 2011, Noumegni *et al* 2021) is not a viable investigative technique, to this purpose.

Temporalis muscle blood flow was measured in few studies in healthy and headache-afflicted volunteers by the Xenon 133 (^{133}Xe) clearance technique (Petersen and Christensen 1973, Jensen and Olesen 1985, Langemark *et al* 1990), which requires injection of tracer depots into the thickest part of the muscle and scintillation detectors over each depot to register the tracer washout. The technique has, obviously, the disadvantage of being invasive and exposing to gamma rays. More recently, magnetic resonance imaging has been employed for diagnostic imaging (Geers *et al* 2005, Veldhoen *et al* 2014). It is, however, quite expensive and can be affected by movement, e.g., by swallowing or coughing, making it unsuitable for continuous bedside monitoring.

A more convenient and noninvasive technique is near-infrared spectroscopy (NIRS), even though it does not measure blood flow: by detecting concentration changes in oxygenated and deoxygenated hemoglobin, NIRS reveals changes in tissue oxygenation and blood volume. This technique is widely adopted to investigate cerebral hemodynamics, with optical probes placed on the forehead as well as all over the skull, by means of dedicated helmets (Chen *et al* 2020). To our knowledge, only two early studies adapted NIRS to investigate hemodynamics of the temporalis muscle which, however, did not consider the possibility of interference from brain hemodynamics (Kim *et al* 1999, Tsukiyama *et al* 1999).

A constant issue with brain monitoring is that the cerebral NIRS measurement may be contaminated by blood flow changes occurring in the more superficial cutaneous and muscular extracranial tissues (Canova *et al* 2011, Schecklmann *et al* 2017). In the same way, the intended monitoring of superficial tissues could be contaminated by deeper ones. Reliable NIRS monitoring of temporalis muscle requires that contribution from more superficial (skin) and more deep tissues (brain) are both excluded from the measurement. While contributions of changes in cutaneous

circulations have been successfully eliminated with different techniques achieved min rhether NIRS measurement can be focused on the temporalis muscle and be unaffected by hemodynamic changes occurring at cerebral level has not been investigated, but is a necessary condition for reliable measurements.

The issue is relevant considering the many clinical conditions that have been related to vascular dysfunctions in this muscle and the lack of alternative monitoring methodologies. The aim of this study was to test the reliability of NIRS monitoring in detecting hemodynamic changes in the temporalis muscle with respect to possible interference from cerebral hemodynamics.

Method

Subjects and ethical approval

Twenty-four (22 ± 2 years; 13 males, 11 females) subjects with no history of bruxism, orofacial pain, or craniomandibular or temporomandibular disorders were enrolled for this study. This study was carried out according to the Declaration of Helsinki and was approved by the University Ethical Committee. All subjects gave their written informed consent before participation.

Monitoring equipment and measurements

The cerebral tissue oxygenation and blood volume were detected by near-infrared spectroscopy (NIRO-200NX, Hamamatsu Photonics, Hamamatsu, Japan), which simultaneously provided conventional Beer-Lambert and spatially resolved spectroscopy parameters, the latter methodology being less affected by changes in cutaneous circulation (Canova *et al* 2011, Messere and Roatta 2013). Spatially resolved spectroscopy measures of tissue oxygenation and blood volume are provided, respectively, by the tissue oxygenation index (TOI), expressed in %, and the tissue hemoglobin index (THI), expressed in arbitrary units (a.u.), whereas Beer-Lambert parameters indicate changes in the concentration of oxyhemoglobin + oxymyoglobin (O_2Hb), deoxyhemoglobin + deoxymyoglobin (HHb) and total hemoglobin (tHb = O_2Hb + HHb), expressed in μmol/L. The device has two probes: the *muscle* probe with an inter-optode distance of 3 cm was placed over the left anterior temporal muscle, while the *brain* probe with an inter-optode distance of 4 cm was placed more medially, over the right prefrontal cortex (Rashid *et al* 2022b). A larger inter-optode distance was chosen for the brain probe to increase the depth of the sample volume, approximately equal to half of the inter-optode distance.

The electromyography was recorded (Quattro, OT Bioelectronics, Torino, Italy; gain 1200; bandwidth 10 – 500 Hz) from the right anterior temporal muscle by means of 2 electrodes (FIAB Spa, Florence, Italy, inter-electrode distance 2.3 cm, inter-electrode axis parallel to the orientation of the muscle fibers, ground electrode sticked to the right ear), aiming to monitor contraction levels during teeth clenching and possible involuntary contraction throughout the experiment.

Clenching force was bilaterally measured through an improved version of a custom made device (Testa *et al* 2015, 2016) based on film sensors (FlexiForce A201 Tekscan, Boston, MA, USA) and visual feedback of the total clenching force (left + right) was provided to the subject to perform constant-force clenching tasks (Testa *et al* 2011).

The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was monitored using a capnograph (Capnostream™ 20p Bedside Patient Monitor with Microstream™ Technology, Oridion Medical, Jerusalem, Israel).

Experimental procedures

The study was performed in a quiet room under constant environmental conditions, with the subject sitting in a comfortable chair, without any visual or auditory disturbances. After a 10-min resting period, necessary to reach stable hemodynamic levels, the subjects performed teeth clenching and hyperventilation, separated by a 10-min interval.

i. Teeth Clenching

The subjects performed a series of teeth clenching at 25, 50, and 75 % of maximum voluntary contraction for 20 s each. The visual feedback of the exerted clenching force was provided on a computer screen and a horizontal cursor was placed to indicate each targeting clenching force level.

ii. Hyperventilation

The subjects were asked to hyperventilate to achieve and maintain for 90 sec the $P_{ET}CO_2$ of 20 mmHg. To this end, they were provided with visual feedback from the display of the capnograph, which was continuously monitoring $P_{ET}CO_2$ from the expiratory flow collected by a nasal cannula, and a horizontal cursor was placed at 20 mmHg indicating the target $P_{ET}CO_2$ (Rashid *et al* 2022a).

Subjects were frequently reminded to maintain the jaw muscles relaxed, particularly during baseline recordings and during hyperventilation.

Data acquisition, processing, and statistical analysis

All signals were continuously digitally sampled (CED Micro 1401 acquisition board and Spike2 ver. 9.15, Cambridge Electronic Design, Cambridge, UK) at 100 Hz and stored on the computer. MATLAB® ver. R2022b (The MathWorks, Natick, MA, USA) was used to analyze signals and IBM® SPSS® Statistics ver. 29 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. The baseline values of all variables were taken as a time average calculated over the 20-s interval preceding the beginning of the task (teeth clenching and hyperventilation), while the task effect was assessed over the last 2-s of teeth clenching and over the last 20-s of hyperventilation. After normality assessment using Kolmogorov-Smirnov test, all variables (ΔO_2Hb, ΔHHb, ΔTOI and ΔTHI) were analyzed. A repeated measures analysis of variance, with factors *probe* (muscle/brain) and *contraction level* (25, 50 and 75 %) was used for teeth clenching analysis. Analysis of hyperventilation, paired student *t*-test was used to test the difference between brain and muscle probes. Data in the Results section are presented as mean ± standard deviation and for all analyses, significance level was set at $p = 0.05$.

Results

No relevant electromyography activation was observed during baseline intervals and during hyperventilation. The time course of hemodynamic responses to teeth clenching and hyperventilation are presented in Figure 1 (left and right), respectively, by average curves of all NIRS variables, obtained from all subjects. Numerical values of observed

changes are reported in Table 1, while statistical significance of differences between muscle and brain variables are reported in Table 2.

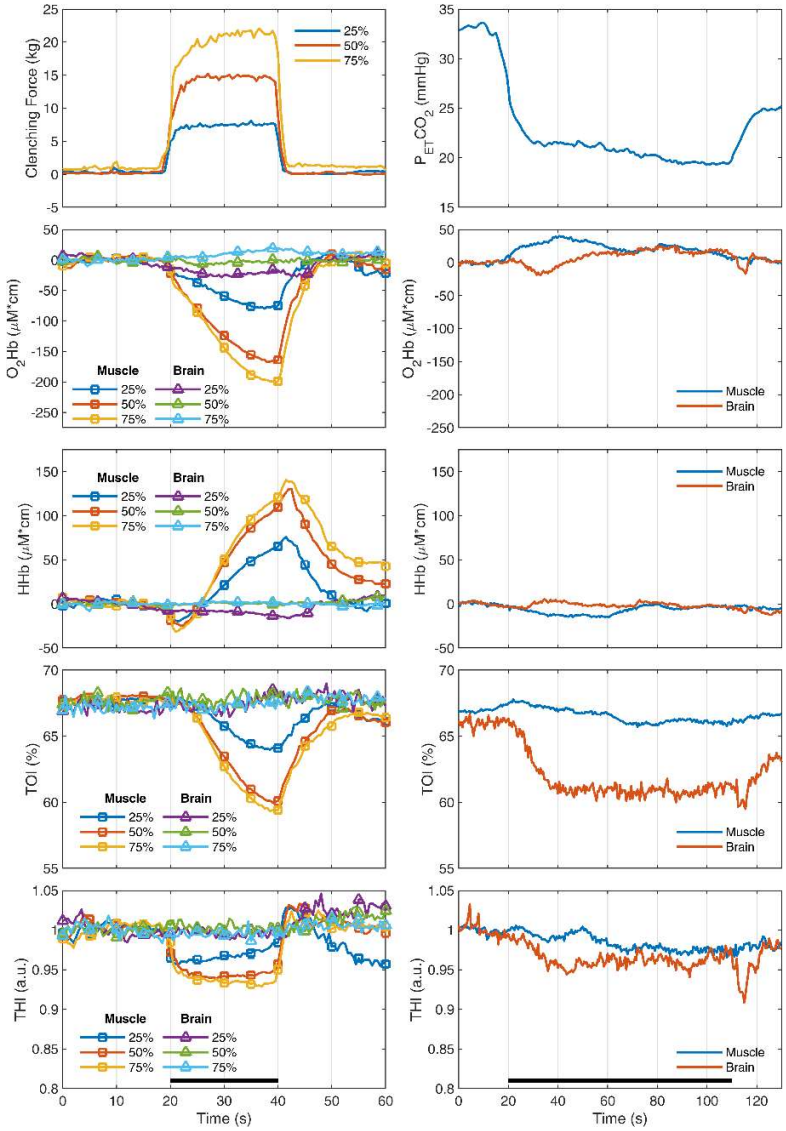

FIGURE 1 Hemodynamic response to teeth clenching (left) and hyperventilation (right). From top to bottom: clenching force (left) and partial pressure of end-tidal CO_2 (right); changes in oxygenated hemoglobin (ΔO_2Hb); and deoxygenated hemoglobin (ΔHHb); tissue oxygenation index (TOI); and tissue hemoglobin index (THI). Each curve represents the average over all subjects (n = 24); responses to the three contraction levels (left only) and the 2 probes (muscle and brain) are superimposed. Black bars at the bottom represent the task duration.

TABLE 1 Changes exhibited by NIRS variables in response to teeth clenching and hyperventilation (mean ± standard deviation; n = 24).

Parameter	Teeth Clenching						Hyperventilation	
	Muscle			Brain			Muscle	Brain
	25% MVC	50% MVC	75% MVC	25% MVC	50% MVC	75% MVC		
ΔO_2Hb (μM*cm)	-76.71 ± 95.31	-166.16 ± 158.28	-199.00 ± 164.98	-18.89 ± 80.99	-0.84 ± 18.59	19.09 ± 30.51	15.25 ± 59.86	15.99 ± 61.98
ΔHHb (μM*cm)	61.98 ± 61.03	104.88 ± 77.24	116.25 ± 82.17	-12.61 ± 48.45	0.09 ± 12.21	1.83 ± 16.67	-3.46 ± 29.33	-2.83 ± 25.09
ΔTOI (%)	-3.60 ± 7.18	-7.18 ± 11.76	-8.26 ± 12.98	0.85 ± 2.07	0.29 ± 1.42	0.35 ± 1.74	-0.92 ± 2.50	-5.08 ± 3.78

ΔTHI (a.u.)	-0.02 ± 0.08	-0.05 ± 0.09	-0.06 ± 0.13	-0.002 ± 0.05	0.002 ± 0.05	-0.002 ± 0.04	-0.03 ± 0.07	-0.04 ± 0.07

Note: MVC – maximum voluntary contraction.

TABLE 2 Significance of main factors (probe location: muscle and brain; contraction level: 25, 50 and 75 % of maximum voluntary contraction) during teeth clenching and muscle versus brain difference during hyperventilation.

Parameter	**Teeth Clenching**			**Hyperventilation**
	Muscle / Brain	Contraction Level	Interaction	Muscle / Brain
O_2Hb (μM*cm)	< 0.001*	0.005*	< 0.001*	0.951
HHb (μM*cm)	< 0.001*	< 0.001*	0.128	0.936
TOI (%)	0.002*	0.010*	0.035*	< 0.001*
THI (a.u.)	0.015*	0.161	0.083	0.647

Note: (*) statistical significance $p = 0.05$.

i. Teeth clenching

The response to the three different contraction levels, are superimposed for both probes with different colors (Fig. 1, left). Signals from the muscle probe exhibit the expected changes that take place during an isometric contraction, namely decrease in oxygenation and O_2Hb and increase in HHb, accompanied by some decrease in blood volume (THI). The effects are generally increasing with the contraction level, although at 50 and 75 % of maximum voluntary contraction the curves are similar. Conversely signals from the brain probe are basically not responding to any level of teeth clenching. Notably all variables exhibit a significant difference between muscle and brain. In particular, the absolute change in tissue oxygenation index (ΔTOI) was -3.60 ± 7.18, -7.80 ± 11.76 and -8.26 ± 12.98 % on the temporal muscle while 0.85 ± 2.07, 0.29 ± 1.42 and 0.35 ± 1.74 % on prefrontal cortex, during 25, 50 and 75 % of maximum voluntary contraction, respectively.

ii. Hyperventilation

Muscle NIRS signals are all unresponsive to hyperventilation as well as O_2Hb, HHb and THI from the brain probe. A distinct response is only exhibited by TOI from the brain probe, in terms of a clear-cut and sustained decrease (-5.08 ± 3.78 %), significantly different ($p < 0.001$) from the responses collected on the muscle (-0.92 ± 2.50 %) (Fig. 1, right).

Discussion

In the present study the use of NIRS for hemodynamic monitoring of the temporalis muscle was tested during isometric muscle contractions (teeth clenching) and hyperventilation. Different levels of muscle contraction produced a marked and progressive decrease in tissue oxygenation (decrease in TOI and O_2Hb and increase in HHb) and blood volume (THI) in a force-level dependent way. In addition, NIRS variables were not affected by hyperventilation, which is known to provoke marked vasoconstriction and decrease in oxygenation of cerebral tissue. Conversely, NIRS monitoring from the forehead correctly detected a consistent TOI decrease during hyperventilation and no changes during teeth clenching. These results demonstrate that NIRS monitoring can be used to reliably detect hemodynamic changes in the temporalis muscle, with no interference from changes in cerebral blood flow.

A number of studies investigating cerebral hemodynamics with functional NIRS evidenced the risk of getting artifacts and disturbances in NIRS signals from the temporalis muscle when optodes are placed on the temple region, as easily occurs when wearing functional NIRS headsets (Schecklmann *et al* 2017, Morais *et al* 2018, Nakajima *et al* 2020).

To our knowledge, NIRS investigation of temporal muscle was implemented only in two early studies, both from the same group (Kim *et al* 1999, Tsukiyama *et al* 1999). However, their observations were limited to the concentration changes of total hemoglobin, taking place during clenching and in the subsequent hyperemic phase. Unfortunately, no information was given about the adopted inter-optode distance, and the possible influence of cerebral hemodynamics on the putative muscle signals was not investigated.

The difficulty of discriminating between superficial and deep tissues is still a major limitation of the NIRS methodology. Vegetative reactions, emotional stimuli and cognitive tasks may affect the superficial extracranial circulation and disturb the assessment of deeper cerebral hemodynamics (Minati *et al* 2011, Canova *et al* 2011, Kirlilna *et al* 2013), just as thermoregulatory changes in the skin may disturb the assessment of hemodynamic changes in skeletal muscles (Messere and Roatta 2013, Grassi and Quaresima 2016). Different methodologies have been devised to limit or exclude the contribution from superficial tissues and focus the measurement in depth. In particular, several studies from our and other groups have pointed-out that spatially-resolved spectroscopy effectively rejects contributions from superficial tissues allowing to more specifically focus the measurement on deeper brain (Quaresima *et al* 2000, Canova *et al* 2011) and muscle tissue (Messere and Roatta 2015, Messere *et al* 2018). The data collected from the brain probe in the present study provide a further confirmation: during hyperventilation, only the spatially-resolved parameter TOI was able to detect the decrease in cerebral oxygenation while the standard Beer-Lambert parameters (O_2Hb and HHb) did not detect significant effects. Investigation of the temporal muscle however presents a further challenge as the measurement needs to be focused on the intermediate muscle layer, i.e., requiring to prevent contributions from the brain, as well as from the skin. This latter requirement can be obtained by adjusting the inter-optode distance. Since, the maximum depth of the NIRS sample volume is roughly equal to half of the inter-optode distance. Distances of 4 cm or greater were recommended for cerebral hemodynamics investigations (Quaresima *et al* 2000). We here maintained a 4-cm distance for the brain probe but adopted a shorter distance (3 cm) for the muscle probe. This choice was effective in making the NIRS measurement on the temporalis muscle independent of cerebral hemodynamics. In fact, a strong stimulus like hyperventilation, which provokes a marked cerebral vasoconstriction and a marked drop in cerebral oxygenation (-5 % in the present study) did not affect the muscle measurement. At the same time, the muscle probe distinctly detected the typical hemodynamic changes of isometric muscle contractions, during teeth clenching. It can be observed in Fig. 1 (left) that the onset of contraction is associated with a sharp reduction of blood volume in the muscle (THI) which slowly recovers at low contraction level (25 % of maximum voluntary contraction), as previously observed (Kim *et al* 1999), but not at higher levels (50 and 75 % of maximum voluntary contraction), which suggests that functional dilatory mechanisms are overcome by intramuscular pressures at high contraction levels.

Muscles of the head present peculiar differences from limb muscles ranging from histology (Stål *et al* 1996), to function (Sciote *et al* 2003), to control of blood perfusion (Rashid and Roatta 2022). However, most functional and hemodynamic studies are carried out on the masseter muscle, while other masticatory and head muscles have been less investigated. The reliability of NIRS monitoring documented in the present study will hopefully promote basic

and clinical investigations of perfusion and oxygenation in the temporalis muscle in physiological and pathological conditions.

Conclusion

By testing the response to teeth clenching and hyperventilation we were able to demonstrate the capacity of NIRS monitoring to focus the measurement of blood volume and tissue oxygenation on the temporalis muscle, with virtually no interference from the brain. Thanks to its non-invasivity and ease of application, this technique will conveniently be adopted in new investigations on the temporalis muscle function, in health and disease.

Authors' Contributions

Both authors contributed equally to this manuscript.

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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