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博 士 論 文

Blood oxygenation of masseter muscle during
sustained elevated muscle activity in healthy
participants
(持続的なヒト閉口筋随意収縮時の
筋内血液酸素動態)

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Blood oxygenation of masseter muscle during sustained elevated muscle activity in healthy participants

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SUMMARY Myofascial pain associated with temporomandibular disorders has often been linked to pathological muscle hyperactivity. As a result, localised disturbances of intramuscular blood flow could lead to a lower level of oxygen distribution, hypoxia and microcirculatory changes. To assess haemodynamic changes in the masseter muscle during sustained elevated muscle activity (SEMA). Sixteen healthy participants performed thirty 1-min bouts of SEMA with intervals of 1-min 'rest' periods between the bouts on a bite force transducer device. The participants completed three sessions with different percentage of their maximal voluntary occlusal bite force (MVOBF): 0% (no task), 10% or 40% MVOBF tasks. The order of the sessions was randomised with 1- to 2-week intervals. Haemodynamic characteristics of the masseter muscle were estimated with use of a laser blood oxygenation monitor. Tissue blood oxygen saturation (StO₂) during SEMA was lower than during rest ($P < 0.001$). The relative changes in

total haemoglobin (Total-Hb) and StO₂ were influenced by condition (SEMA and rest) and with interactions between condition and session (0%, 10% and 40% MVOBF tasks). These results suggest that SEMA may lead to hypoxia in the masseter muscle and that the haemodynamic characteristics and muscle symptoms depend on the magnitude of muscle contractions. Overall, the present findings may help to provide better insights into relationships between jaw muscle activity, haemodynamic changes and symptom developments with implications for clinical conditions such as bruxism characterised by different levels of tooth-grinding and tooth-clenching muscle activity.

KEYWORDS: blood oxygenation, masseter muscle, maximal voluntary bite force, sustained elevated muscle activity

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Introduction

Myofascial pain and tension-type headache (TTH) associated with temporomandibular disorders (TMD) have traditionally been linked to hyperactivity or abnormal contraction of craniofacial muscles, because a frequent clinical observation has been that many of such patients exhibit a tendency to clench or grind their teeth, that is bruxism (1, 2). Bruxism is a

repetitive jaw muscle activity characterised by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible (3). Bruxism has two distinct circadian manifestations: it can occur during sleep (sleep bruxism) or during wakefulness (awake bruxism) (3). The potential harmful effects of bruxism on the masticatory system have often been discussed especially in relation to tooth wear, dental erosion, gastroesophageal reflux, periodontal health and/or

bone damage, temporomandibular joint and craniofacial muscle symptoms (4). Indeed, a large number of papers have described muscle pain and fatigue evoked during experimental tooth-grinding/tooth-clenching tasks (5–8). These studies, which used various tooth-grinding/tooth-clenching tasks, for example short-duration, high-intensity clenching tasks or long-duration, low-intensity clenching tasks, have collectively shown that transient (hours–days) masticatory muscle pain and fatigue can be produced but that the symptoms quickly (within days) disappear or decrease.

Later, it has been suggested that chronic muscle pain could be related to localised disturbances of intramuscular blood flow leading to a lower level of oxygen distribution, hypoxia and microcirculatory changes that may involve subclinical inflammatory changes in the affected tissue facilitating pain (9–11). Using near-infrared spectroscopy, Delcanho *et al.* showed less perfusion in the masseter muscle immediately after submaximal isometric contractions in patients with chronically painful masticatory muscles than in healthy participants (12).

However, the relationship between muscle hyperactivity, haemodynamics and myofascial TMD pain and TTH is still unclear because it has been technically difficult to assess the intramuscular changes in patients with myofascial pain or headache.

The aim of this study was to assess haemodynamic characteristics in the masseter muscle during sustained elevated muscle activity (SEMA) in healthy participants. The hypothesis was that there would be significant differences between no, light or moderate SEMA in terms of the haemodynamic responses as well as the development of muscle symptoms such as fatigue and pain.

Materials and methods

Participants

Sixteen healthy participants, seven male and nine female, mean age 26.3 ± 5.1 (mean $\pm$ s.d.) years, range 21–40 years, were recruited at Aarhus University, Denmark. Questionnaire-based exclusion criteria were the following: ongoing pain, chronic pain during the last 6 months, systemic diseases (e.g. metabolic diseases, neurogenic diseases and cardiovascular disorders), previous radiotherapy or chemotherapy, intake of any medicine affecting the nervous system in the

24 h before the experiments, physical or mental disorders, for example fibromyalgia syndrome (FMS), or psychogenic illnesses. The participants had no signs or symptoms of TMD according to the DC/TMD screener (13). None of the participants took medication that could influence psychological and/or cardiovascular responses. Informed consent was obtained from each participant, and the experimental protocol was approved by the local ethics committee in Region Midt (Denmark) in accordance with the Declaration of Helsinki.

As an additional experiment, six other healthy participants, four male and two female, mean age 30.2 ± 1.9 (mean $\pm$ s.d.) years, range 28–33 years, were recruited in a same way as described above to assess stress levels in a control session without any SEMA tasks.

Study design

The participants sat on a comfortable chair in a temperature-controlled room (approx. 24 °C) and in three sessions performed three different kinds of SEMA tasks: 0%; (no task), 10% and 40% of their maximal voluntary occlusal bite force (MVOBF) tasks. Perceived levels of pain, fatigue and unpleasantness in the right masseter muscle during the sessions with 10% and 40% MVOBF tasks were registered using 0–10 numerical rating scale (NRS). Intramuscular blood oxygenations (BO) in the right masseter muscle were recorded continuously during all the different sessions (0%, 10% and 40% MVOBF tasks).

Sustained elevated muscle activity

First, the participants were asked to bite as hard as they possibly could on a bite force device to record the MVOBF before starting the session (14). The MVOBF was measured in Newton (N) with a bite force transducer ($41.0 \times 12.0 \times 5.0$ mm, length $\times$ width $\times$ height, Aalborg University, Aalborg, Denmark) inserted between the first or second molars on the right side. The participants were encouraged to achieve their maximum for 2–3 s and then to relax. The analogue output of the amplifier was connected to an analogue-to-digital converter*, and the bite

*BNC-2090; National Instruments, Austin, TX, USA.

force signal was analogue-to-digital converted, sampled at 2 kHz and stored on a personal computer for later analyses. To hold the centre of the bite force transducer between the upper and lower teeth, a layer of acrylic resin with 2-mm thickness and silicone impression material[†] were added on the blocks and fitted to the occlusion. The assessment was carried out in triplicate, and the mean was calculated. The mean value of the three MVOBF assessments served to define the 10% and 40% MVOBF tasks.

The participants were then instructed to observe the bite force display as a feedback to ensure that the biting force was kept constant during the session. The examiner continuously supervised and encouraged the participants to maintain the target force level. The participants underwent thirty 1-min bouts of SEMA with intervals of 1 min of rest periods (Rest) between the bouts. Each session lasted about 1 h. The participants performed three sessions with different SEMA tasks: 0% (no task), 10% and 40% MVOBF with an interval of about 1–2 weeks between sessions. During the 0% MVOBF task session, the participants were instructed to relax their face and jaw muscles while sitting on the dental chair for 1 h. The sequence of the three sessions was decided at random to avoid sequence effects of the SEMA tasks.

Assessment of pain, fatigue and unpleasantness

The participants were asked to stay as relaxed as possible with the head supported by the headrest of the dental chair during all sessions in order to minimise artefacts by head movements. The participants were also asked to fill out a paper form in which they rated their pain, fatigue and unpleasantness intensity in the right masseter muscle on three separate 0–10 NRS with the '0' extreme representing 'no pain, no fatigue or no unpleasantness' and the '10' representing 'the most imaginable pain, fatigue or unpleasantness'. The participants were asked to give scores on the NRS at every Rest in the 10% and 40% MVOBF task sessions. The participants assessed the levels of their pain, fatigue and unpleasantness in NRS corresponding to Rest ('how much pain/fatigue/unpleasantness do you feel right now?'). The registrations of the NRS of pain during SEMA were also assessed during the

Rest immediately following the SEMA as a 'recall' assessment ('how much pain did you feel while you were biting?'). In the 0% MVOBF task session, no NRS scores were obtained throughout the session, but all participants were confirmed to be pain-, fatigue- and unpleasantness-free before starting the session; that is, all NRS scores were 0. The participants were instructed to report if any changes in these three aspects (pain/fatigue/unpleasantness) happened during the 0% MVOBF task session.

Assessment of intramuscular blood oxygenation

Haemodynamic characteristics were measured from the right masseter muscle with the use of a near-infrared spectroscopy laser blood oxygenation monitor[‡] during the three different SEMA tasks.

The BOM recording device had one light source and two detectors. The distance between the light source and detector 1 was 1 cm and 2 cm to detector 2 so that BO between the depth of 1 cm and 2 cm from the skin surface could be measured (Fig. 1). The device was placed in the middle of the right masseter muscle parallel to the main directions of muscle fibres as determined from palpation of the muscles. The detector 1 which was closer to the light source was

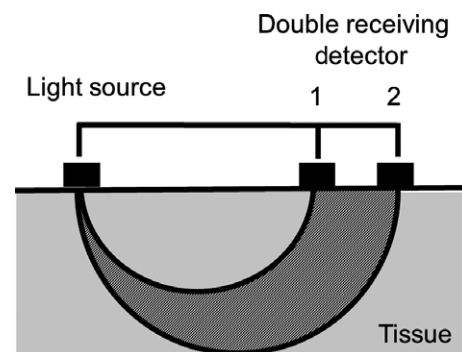


Fig. 1. Principle of blood oxygenation monitoring. The figure shows schematic representation of the blood oxygenation monitor set-up. The lights from the 'light source' are scattered and absorbed in the tissue, and a part of the scattered lights is detected by the 'Detector 1' and 'Detector 2'. The absorption in the tissue is mainly caused by haemoglobin, and the absorption spectra of oxygenated haemoglobin and deoxygenated haemoglobin are different. The oxygenated blood volume and deoxygenated blood volume are calculated from the different change in the detected laser light intensity.

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put close to the most bulky point of the muscle which was found in the lower 1/3 of the muscle determined by manual palpation while asking the participant to clench for a few seconds.

The following parameters were derived from the BOM: oxygenated haemoglobin (Oxy-Hb) and deoxygenated haemoglobin (Deoxy-Hb). Total haemoglobin (Total-Hb) and tissue blood oxygen saturation (StO₂) were calculated based on Oxy-Hb and Deoxy-Hb (8).

Additional experiment

The participants in the additional experiment ($n = 6$) were asked to keep their jaws relaxed and to avoid movement for 61 min under monitoring and recording BO in the right masseter muscle and to fill out self-report questionnaires of 0–10 NRS scores of perceived mental stress levels with the '0' extreme representing 'not stressed at all' and the '10' representing 'the most stressed you can imagine' before starting BO recording as baseline and at every 15 min. The systolic and diastolic blood pressure (mmHg) and pulse (bpm) were recorded before starting BO recording as baseline and at every 15 min with an automatic blood pressure monitor[§].

Statistical analyses

All data were expressed as mean $\pm$ s.e.m. (standard error of the mean). The triplicate and averaged assessments of the MVOBF were tested by two-way analysis of variance (ANOVA) with gender (female and male) as an independent factor and session (10% and 40% MVOBF tasks) as a dependent factor. The results of the actual bite force during the SEMAs were averaged for each 1-min SEMA, and subsequently, five epochs of the 1-min average were averaged. They were then compared by three-way ANOVA with the following factors: gender (female and male) as an independent factor, session (10% and 40% MVOBF tasks) and time (six blocks of 10 min from 0 to 60 min) as dependent factors.

Multivariate analysis of variance (MANOVA) was used to test the pain NRS scores with the following factors: gender (female and male) as an independent factor,

condition (SEMA and Rest), session (10% and 40% MVOBF tasks) and time (0–60 min) as dependent factors. MANOVAS were also used to test the data of fatigue and unpleasantness NRS scores with the following factors: gender (female and male) as an independent factor, force (10% and 40% MVOBF tasks) and time (0–60 min).

For the analysis of blood oxygenation parameters, the baseline absolute values of Oxy-Hb, Deoxy-Hb, Total-Hb and StO₂, which were defined as a 1-min period before the onset of the SEMA tasks (0%, 10% and 40% MVOBF tasks), were tested by two-way ANOVA with these factors: gender (female and male) as an independent factor and session (0%, 10% and 40% MVOBF tasks). Then, all data were averaged for every block of 10 min (intercalated periods of 5 min for SEMA and 5 min for Rest) to observe changes over time. Five-second transition periods from Rest to SEMA and from SEMA to Rest were excluded for all the periods. Each parameter was characterised as a relative change from the baseline value. MANOVA was used to test the BO data with the following factors: gender (female and male) as an independent factor, condition (SEMA or Rest), session (0%, 10% and 40% MVOBF tasks) and time (six blocks of 10 min from 1 to 60 min).

Regarding the results of the additional experiment, blood oxygenation parameters were averaged for 5 min and calculated to relative changes from the first 1-min values as the baseline. Each parameter was tested by one-way ANOVA with a factor of time (1 to 60 min). Systolic, diastolic blood pressure and pulse data were also characterised as a relative change from the baseline value and tested by one-way ANOVA with a factor of time (baseline, 15 min, 30 min, 45 min and 60 min). The NRS scores for mental stress were averaged and tested by one-way ANOVA with a factor of time (baseline, 15 min, 30 min, 45 min and 60 min).

Post hoc comparisons were performed using Tukey HSD *post hoc* test with correction for multiple comparisons with a limitation to two-way interactions. There were no missing data. For all tests, *P* values less than 0.05 were considered to be statistically significant. The STATISTICA software[¶] was used for all analyses.

[§]Omron M6 HEM-7001-E; OMRON HEALTHCARE Co., Ltd, Kyoto, Japan.

[¶]StatSoft, Tulsa, OK, USA.

Results

Experimental SEMA task

All participants completed the SEMA tasks in the two active sessions (10% and 40% MVOBF tasks). The average MVOBF was 279.9 ± 38.5 N (male: 320.0 ± 55.2 N, female: 241.0 ± 49.2 N). As a result of the MANOVA, there were no significant main effects of gender ($P > 0.267$), session ($P > 0.952$) and no significant interaction ($P = 0.227$).

The average 10% MVOBF corresponded to 28.0 ± 16.2 N (male: 34.2 ± 6.0 N, female: 22.1 ± 4.8 N), and the average 40% MVOBF corresponded to 111.9 ± 60.3 N (male: 119.4 ± 21.4 N, female: 104.2 ± 20.8 N). The mean percentage of the actual bite force of the 10% MVOBF task was $10.1 \pm 0.1\%$ (female: $10.1 \pm 0.07\%$, male: $10.0 \pm 0.03\%$), and for the 40% MVOBF task, it was $32.7 \pm 0.4\%$ (female: $33.5 \pm 0.4\%$, male: $31.7 \pm 0.5\%$). There was no significant main effect of gender ($P = 0.579$) or time ($P = 0.077$). However, there was a significant main effect of session ($P < 0.001$), and the post hoc test showed (as expected) that all 40% MVOBF tasks were significantly higher than the 10% MVOBF tasks ($P < 0.001$).

Pain, fatigue and unpleasantness

Table 1 shows the peak and peak time of the different NRS scores during 10% and 40% MVOBF tasks.

NRS scores of pain, fatigue and unpleasantness in the masseter muscles are shown for each session (10% and 40% MVOBF tasks) in Fig. 2. *P*-values of

the MANOVA are shown in Table 2. NRS scores of pain, fatigue and unpleasantness were significantly dependent on session (10% and 40% MVOBF tasks) and time ($P < 0.001$). There were also significant interactions between session and time ($P < 0.001$). The *post hoc* tests of the interaction (session $\times$ time) indicated that the NRS scores of pain, fatigue and unpleasantness in the 40% MVOBF task were significantly higher than the NRS values in the 10% MVOBF task all time points after six min ($P < 0.001$, Fig. 2).

As for gender differences, there were significant interactions between gender and time for pain, fatigue and unpleasantness ($P < 0.001$). The post hoc tests of these interactions (gender $\times$ time) showed that the NRS scores from women were significantly higher than those from men at 52 min (after 26 SEMA) and 60 min (after 30 SEMA) ($P < 0.008$) for fatigue; only at 56 min (after 28 SEMA) for unpleasantness ($P = 0.014$); and at 56, 58 and 60 min (after 28, 29 and 30 SEMA) for pain ($P < 0.017$).

Intramuscular blood oxygenation data

Baseline values were calculated as the means of a 1-min period before the onset of the SEMA task. ANOVA showed that there were no significant differences in any of the BOM outcome parameters within baseline absolute values ($P > 0.055$).

The relative changes in Oxy-Hb, Deoxy-Hb, Total-Hb and StO₂ were calculated and analysed. Significant haemodynamic changes in the right masseter muscle were demonstrated in the experimental sessions (Fig. 3). *P*-values of MANOVA are shown in Table 2. The relative change in Oxy-Hb was significantly affected

Table 1. Peak of numerical rating scale (NRS) scores for pain, fatigue and unpleasantness

	Peak of NRS scores			
	Pain (SEMA)	Pain (Rest)	Fatigue	Unpleasantness
10% MVOBF				
Peak time	60 min (NRS:2.3 $\pm$ 0.5)	60 min (NRS:1.6 $\pm$ 0.5)	60 min (NRS:3.3 $\pm$ 0.6)	54 min (NRS:2.5 $\pm$ 0.6)
Peak score				
40% MVOBF				
Peak time	54 min (NRS:5.7 $\pm$ 0.7)	60 min (NRS:4.2 $\pm$ 0.6)	60 min (NRS:6.6 $\pm$ 0.6)	60 min (NRS:6.3 $\pm$ 0.6)
Peak score				

This table shows the peak scores (means $\pm$ standard error of mean; $n = 16$) and peak times of the numerical rating scale (NRS) for pain during the sustained elevated muscle activity (SEMA) and between the SEMA (Rest). Moreover, peak scores and peak times of the NRS values of fatigue and unpleasantness are also presented.

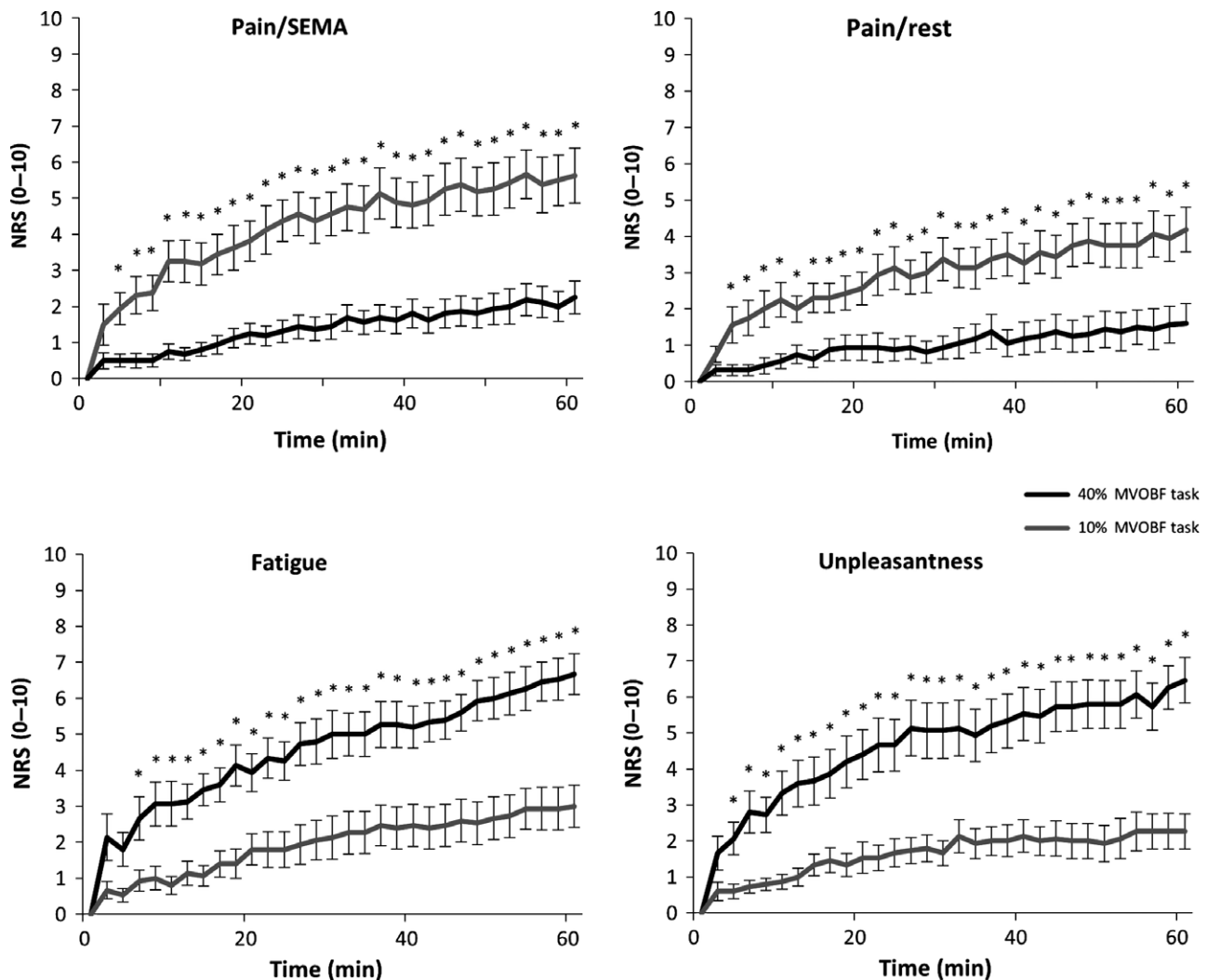


Fig. 2. Numerical rating scale scores for pain, fatigue and unpleasantness. The figure shows the numerical rating scale for pain, during the sustained elevated muscle activity (SEMA) and between the SEMA (Rest), fatigue and unpleasantness during Rest in the right masseter muscle. Each value represents the means $\pm$ standard error of mean ($n = 16$). Significant differences ($P < 0.05$) are indicated (*).

by time ($P < 0.001$), but there were no significant interactions ($P > 0.310$). The relative change in Deoxy-Hb was significantly influenced by condition, time and with interactions between condition and session ($P < 0.001$). The results of the post hoc test for this interaction (condition $\times$ force) showed that the relative change in Deoxy-Hb during SEMA was significantly higher than during Rest for both the 10% MVOBF task ($P = 0.012$) and 40% MVOBF task ($P < 0.001$), but there was no significant difference between the 10% and 40% MVOBF tasks during SEMA ($P = 0.077$) and during Rest ($P = 0.970$). The relative changes in Total-Hb were significantly influenced by time ($P < 0.001$) and interactions between

condition and force ($P = 0.033$). The *post hoc* test of the interaction (condition $\times$ force) indicated that the relative change in Total-Hb during SEMA in the 40% MVOBF task was significantly higher than during Rest ($P = 0.015$), even though there was no significant difference between SEMA and Rest in the 10% MVOBF task ($P = 0.859$). The relative change in Total-Hb during SEMA in the 40% MVOBF task was also significantly higher than during SEMA in the 10% MVOBF task ($P = 0.015$), but there was no significant difference during Rest between the 10% and 40% MVOBF tasks. The relative change in StO₂ was significantly influenced by condition ($P < 0.001$), time ($P < 0.001$) and interactions between condition and session

Table 2. *P*-value of multivariate analysis of variance for blood oxygenation, pain, fatigue and unpleasantness

	Gender		Condition		Session		Time					
Blood oxygenation												
Oxy-Hb		0.408		0.126		0.957		<0.001*				
Deoxy-Hb		0.308		0.005*		0.093		<0.001*				
Total-Hb		0.863		0.061		0.364		<0.001*				
StO ₂		0.098		<0.001*		0.675		<0.001*				
Pain		0.523		0.001*		<0.001*		<0.001*				
Unpleasant		0.682		–		<0.001*		<0.001*				
Fatigue		0.677		–		0.001*		<0.001*				
	Interaction											
	Gender × Condition		Gender × Session		Condition × Session		Gender × Time		Condition × Time		Session × Time	
Blood oxygenation												
Oxy-Hb	0.651		0.478		0.360		0.730		0.460		0.731	
Deoxy-Hb	0.316		0.975		<0.001*		0.272		0.798		0.531	
Total-Hb	0.370		0.604		0.032*		0.806		0.910		0.436	
StO ₂	0.806		0.650		0.002*		0.006*		0.434		0.940	
Pain	0.871		0.615		0.003*		<0.001*		<0.001*		<0.001*	
Unpleasant	–		0.831		–		<0.001*		–		<0.001*	
Fatigue	–		0.616		–		<0.001*		–		<0.001*	

This table shows *P* values for the results of multivariate analysis of variance (MANOVA) with condition (during the sustained elevated muscle activity (SEMA), and Rest: between the SEMA), force (0%, 10% and 40% MVOBF tasks) and time. Significant difference by three-way ANOVA or two-way ANOVA ($P < 0.050$) is indicated (*). Oxy-Hb = relative change in oxygenated haemoglobin; Deoxy-Hb = relative change in deoxygenated haemoglobin; Total-Hb = relative change in total haemoglobin; StO₂ = relative change in tissue blood oxygen saturation.

($P = 0.002$) and between gender and time ($P = 0.006$). The results of the *post hoc* test with the interaction (condition × session) showed that the relative changes in StO₂ during SEMA were significantly lower than Rest for both the 10% MVOBF task ($P = 0.010$) and 40% MVOBF task ($P < 0.001$) although both of them increased from the baseline. On the other hand, there were no significant differences in the relative changes in StO₂ between the 10% and 40% MVOBF tasks during SEMA ($P = 0.781$) and Rest ($P = 0.960$). The *post hoc* test with the interaction (gender × time) did not indicate significant differences between gender until 20 min ($P > 0.141$), but the relative change in StO₂ in women was significantly higher than that in men from 30 min to 60 min ($P < 0.020$).

Additional experiment

One-way ANOVA tests for the relative change in Oxy-Hb, Total-Hb and StO₂ indicated a significant main effect of time ($P < 0.036$), but not for Deoxy-Hb. Post

hoc tests showed that the relative change in Oxy-Hb and StO₂ was significantly higher after 35 min than the baseline values ($P < 0.018$). No other relevant significant differences were found.

The peak NRS score of mental stress was 2.7 ± 0.3 which occurred after 60 min. One-way ANOVA for the self-report questionnaire of NRS scores for mental stress indicated a significant main effect of time ($P < 0.001$), and the *post hoc* test showed the NRS scores were significantly higher after 30 min than the baseline ($P < 0.002$).

One-way ANOVA for the relative changes in systolic and diastolic blood pressure and pulse indicated that there was no significant main effect of time ($P > 0.407$).

Discussion

Overall, the main finding of this study was that the several haemodynamic parameters were significantly affected by time, conditions and sessions in response to SEMA. It suggests that different levels of

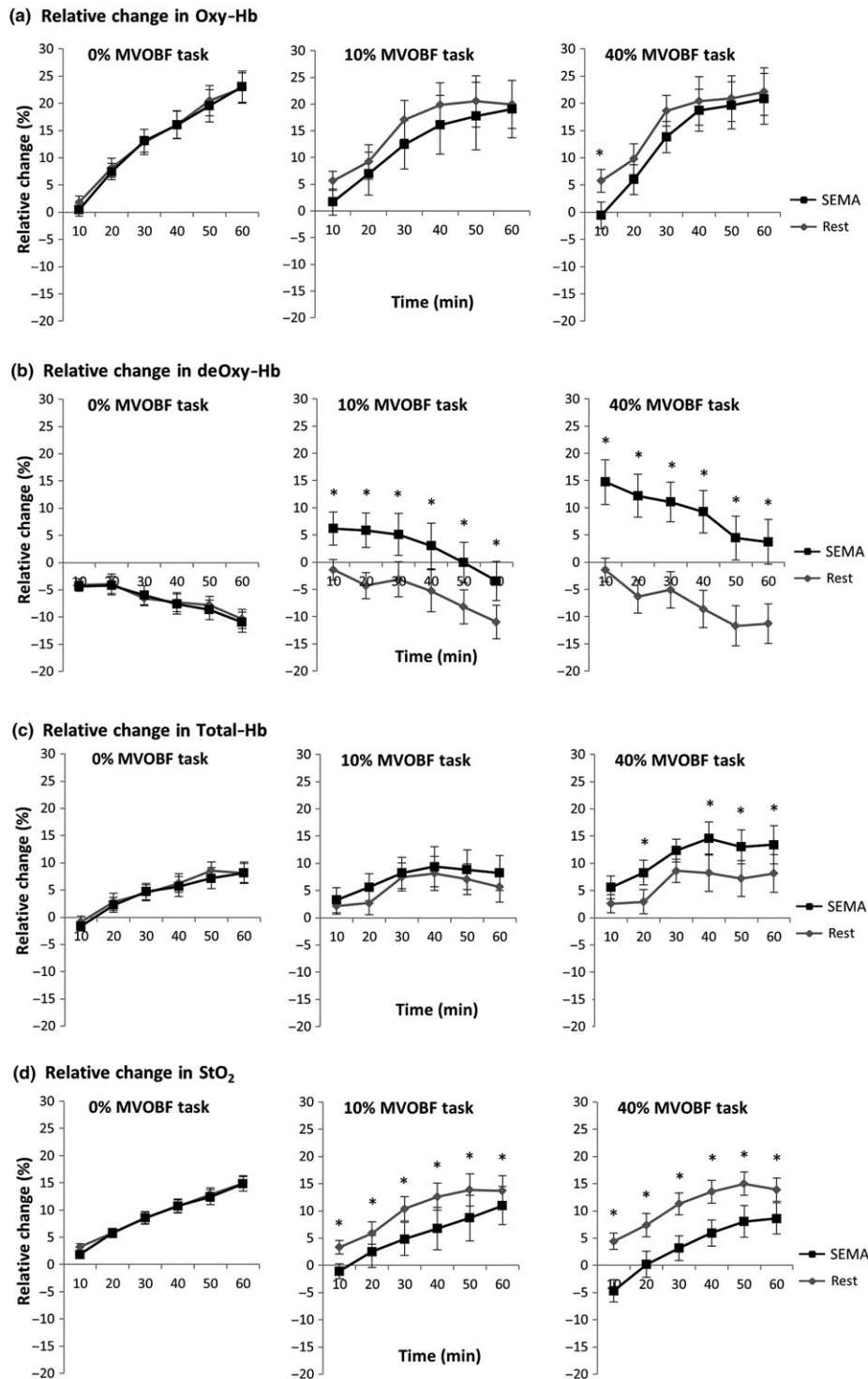


Fig. 3. Relative changes of oxygenated hemoglobin (OxyHb) (a), deoxygenated hemoglobin (deOxyHb) (b), total hemoglobin (TotalHb) (c) and tissue blood oxygen saturation (StO₂) (d). The figure shows the effects on the relative changes BOM parameters in right masseter muscle of the sustained elevated muscle activity (SEMA) task compared to Rest periods in three different intensity task sessions (0%, 10% and 40% MVOBF tasks). Each value represents the means $\pm$ standard error of mean ($n = 16$). For the analysis of blood oxygenation, all data were averaged every 10-min block (intercalated 5 min for SEMA and 5 min for Rest). Significant condition differences ($P < 0.002$) are indicated (*).

contraction of the masseter muscle may lead to different haemodynamic characteristics that may imply different muscle symptoms.

Maximal voluntary occlusal bite force levels

The average MVOBF in this study was about 151% higher than the results by Takeuchi *et al.* (8). The explanation for this difference is related to the placement of the bite force transducer which in the present study was placed between the molars and in the Takeuchi *et al.* study between the incisors. This observation is in accordance with several other studies (15, 16). For example, Roldan *et al.* showed that the molar bite force was higher than the incisor bite force (15). Another study by Dawson *et al.* showed about 90% higher MVOBF values than in the present study (17). This difference may be caused by differences in the interocclusal distance. A previous study by Arima *et al.* showed that the MVOBF decreased with increased interocclusal distance between 8 mm to 12 mm (18). The height of the bite force transducer used for our present study was 5 mm, and two layers of acrylic resin with 2 mm thickness were added on to the blocks. The silicone impression material was also added on to the block to keep the same biting position and in order to protect the teeth, but did not interfere with the total thickness of the bite force transducer.

NRS scores for pain, fatigue and unpleasantness

NRS scores of pain, fatigue and unpleasantness all showed similar trends over time which was corroborated by the MANOVA results indicating significant effects of session (10% and 40% MVOBF tasks) and time with interactions between session and time. Pain intensity and fatigue in response to the SEMA task were also assessed by Dawson *et al.* (17). Pain intensity increased significantly over time in the SEMA task sessions for this previous study (17), in agreement with our results. In contrast, for gender difference, there were significant interactions between gender and time for pain, fatigue and unpleasantness in the present study; however, there were no significant differences between genders in terms of pain intensity and fatigue in the study by Dawson *et al.* (17). It should be noted that the present study was not specifically powered to address the issue of

potential gender differences in muscle symptoms following SEMA and more research will be needed to better understand the clinical significance of findings from experimental studies.

Pain and fatigue in the masseter and temporalis muscle following SEMA were also assessed by Takeuchi *et al.* (8). In this previous study during a 10% MVOBF task for 120 min, the pain in the masseter and temporalis muscles reached a peak at 110 min (NRS 2.1 ± 2.8) (8). The results of Takeuchi *et al.* on peak NRS score in the masseter muscle during a 10% MVOBF task is similar to our results assessed in the masseter muscle using the same 10% MVOBF but lower than during the 40% MVOBF task. Our results showed significantly different peak NRS scores for pain in the masseter muscle during the SEMA task with 10% and 40% MVOBF tasks.

Relative changes in haemodynamic parameters

The relative change in Total-Hb during SEMA was significantly higher than during Rest in the 40% MVOBF task, even though there was no significant difference between during SEMA and during Rest in the 10% MVOBF task. This may be because the relative change in Total-Hb during SEMA in the 40% MVOBF task was significantly higher than in the 10% MVOBF although there was no significant difference between 10% and 40% MVOBF tasks during Rest. Changes in intramuscular blood flow during muscle contraction rely on the balance between the increases in intramuscular pressure and the increase in perfusion pressure that is associated with the force and duration of contraction (19), and the local metabolic changes cause relaxation of the resistance vessels (12, 20). A previous study by Curtis *et al.* showed that there was no linear increase in blood flow with an increase in the bite force level (25%, 50% and 100% MVOBF tasks) with the use of a single-fibre probe laser Doppler flowmeter (21). However, our results cannot be compared directly with these data because of the difference in methods used to assess blood flow.

Total-Hb and StO₂ during Rest also increased from the baseline. This might be the effect of a reactive hyperaemia, which has been thought to be associated with SEMA, indicating a compensation for an insufficient blood flow during SEMA (9). The reactive hyperaemia also can be found in limb muscles after submaximal isometric contractions (22, 23).

The present results showed that there were also increases in the relative changes in Oxy-Hb, Total-Hb and StO₂ in the 0% MVOBF task sessions (no task). In the additional experiment that followed the same design as the 0% MVOBF task session (no task), we were able to replicate the same main effects of time in the relative change in Oxy-Hb, Total-Hb and StO₂. Moreover, the relative changes in Oxy-Hb and StO₂ were significantly higher after 35 min compared to baseline. NRS scores of the self-report questionnaire for mental stress was also slightly but significantly increased after 30 min (1.3 ± 0.5 on 0–10 NRS scale) even though systolic and diastolic blood pressure and pulse rate were stable during the whole experiment. Hidaka *et al.* suggested that a weaker but longer stressor can increase sympathetic nervous activity without causing an increase in heart rate and blood pressure, resulting in an increase in haemodynamics (24). The haemodynamic changes that were observed in our present study may also be caused by the weak mental stress that was the result of the present study experimental set-up and it was lower level than the one reported by Hidaka *et al.* (24).

There were no significant main effects of gender for the relative changes in any of the haemodynamic parameters. However, the relative changes in StO₂ demonstrated a significant interaction between gender and time (Table 2), and women had significantly higher values than men from 30 min to 60 min. In a similar way, a previous study showed that there were interactions between gender, time (0–120 min) and day (totally 3 days) for Oxy-Hb, Total-Hb and StO₂ in accordance with the results (17). Even though the specific mode of the SEMA task was different between the studies, a continuous SEMA task (8) versus a repeated SEMA task in the present study, the results of StO₂ are in agreement with the previously reported results (8). However, as indicated above, the present study was not designed or powered to demonstrate gender differences in haemodynamic parameters, and further studies will be needed to address this in more detail, in particular in relation to development of pain and fatigue.

The relative changes in Oxy-Hb, Total-Hb and StO₂ increased over time. All NRS scores for pain, fatigue and unpleasantness also increased over time. These results seem not to agree with the notion that muscle pain could be related to localised disturbances of intramuscular blood flow leading to a lower level of

oxygen distribution and hypoxia. Østergaard *et al.* hypothesised that capillary flow disturbances, rather than arteriolar compressions or vasospasms, and tissue hypoxia, rather than ischaemia, may be related to complex regional pain syndrome (11); therefore, capillary flow disturbances might also play an important role in muscle pain.

The near-infrared spectroscopy used in this present study unavoidably included signals from the skin vasculature and blood vessels (12). Despite this limitation of the method, we still believe that the method used in this experiment was able to capture haemodynamic changes in the muscle tissue as it has been suggested in previous studies (8, 12).

Conclusions

Within the limitations of this study, the results suggest that different levels of contraction of the masticatory muscles may lead to different haemodynamic characteristics and muscle symptoms such as pain and fatigue. Overall, the present findings may help to provide better insights into relationships between jaw muscle activity, haemodynamic changes and symptom developments with implications for clinical conditions such as bruxism characterised by different levels of tooth-grinding and tooth-clenching.

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References

1. Friction JR, Kroening R, Haley D, Siegert R. Myofascial pain syndrome of the head and neck: a review of clinical characteristics of 164 patients. *Oral Surg Oral Med Oral Pathol.* 1985;60:615–623.
2. Bush FM, Whitehill JM, Martelli MF. Pain assessment in temporomandibular disorders. *Cranio.* 1989;7:137–143.
3. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ *et al.* Bruxism defined and graded: an international consensus. *J Oral Rehabil.* 2013;40:2–4.
4. Lobbezoo F, Lavigne GJ. Do bruxism and temporomandibular disorders have a cause-and-effect relationship? *J Orofac Pain.* 1997;11:15–23.

5. Arima T, Svensson P, Arendt-Nielsen L. Experimental grinding in healthy subjects: a model for postexercise jaw muscle soreness? *J Orofac Pain*. 1999;13:104–114.
6. Svensson P, Burgaard A, Schlosser S. Fatigue and pain in human jaw muscles during a sustained, lowintensity clenching task. *Arch Oral Biol*. 2001;46:773–777.
7. Farella M, Soneda K, Vilmann A, Thomsen CE, Akke M. Jaw muscle soreness after tooth-clenching depends on force level. *J Dent Res*. 2010;89:717–721.
8. Takeuchi T, Arima T, Ernberg M, Yamaguchi T, Ohata N, Svensson P. Symptoms and physiological responses to prolonged, repeated, low-level tooth clenching in humans. *Am Headache Soc*. 2015;55:381–394.
9. Monteiro AA, Kopp S. Estimation of blood flow by ¹³³Xe clearance in human masseter muscle during rest, endurance of isometric contraction, and recovery. *Arch Oral Biol*. 1988;33:561–565.
10. Larsson SE, Bodegard L, Henriksson KG, Oberg PA. Chronic trapezius myalgia: morphology and blood flow studied in 17 patients. *Acta Orthop Scand*. 1990;61:394–398.
11. Østergaard L, Terlelsen AJ, Finnerup NB, Knudsen L, Drabek KR, Jespersen SN *et al.* Capillary dysfunction and impaired tissue oxygenation in complex regional pain syndrome: a hypothesis. *Pain*. 2014;155:1922–1926.
12. Delcanho RE, Kim YJ, Clark GT. Haemodynamic changes induced by submaximal isometric contraction in painful and non-painful human masseter using near-infra-red spectroscopy. *Arch Oral Biol*. 1996;41:585–596.
13. Gonzalez YM, Schiffman E, Gordon G, Seago B, Truelove EL, Slade G *et al.* Development of a brief and effective temporomandibular disorder pain screening questionnaire: reliability and validity. *J Am Dent Assoc*. 2011;142:1183–1191.
14. Dawson A, List T, Ernberg M, Svensson P. Assessment of proprioceptive allodynia after tooth-clenching exercises. *J Orofac Pain*. 2012;26:39–48.
15. Roldán S, Buschang PH, Isaza Saldarriaga JF, Throckmorton G. Reliability of maximum bite force measurements in age-varying populations. *J Oral Rehabil*. 2009;36:801–807.
16. Gonzalez Y, Iwasaki LR, McCall WD, Ohrbach R, Lozier E, Nickel JC. Reliability of EMG activity versus bite-force from human masticatory muscles. *Eur J Oral Sci*. 2011;119:219–224.
17. Dawson A, Ghafouri B, Gerdle B, List T, Svensson P, Ernberg M. Pain and intramuscular release of algescic substances in the masseter muscle after experimental tooth-clenching exercises in healthy subjects. *J Orofac Pain*. 2013;27:350–360.
18. Arima T, Takeuchi T, Honda K, Tomonaga A, Tanosoto T, Ohata N *et al.* Effects of interocclusal distance on bite force and masseter EMG in healthy participants. *J Oral Rehabil*. 2013;40:900–908.
19. Sjøgaard G, Savard G, Juel C. Muscle blood flow during isometric activity and its relation to muscle fatigue. *Eur J Appl Physiol Occup Physiol*. 1988;57:327–335.
20. Monteiro AA, Svensson H, Bornmyr S, Arborelius M, Kopp S. Comparison of ¹³³Xe clearance and laser Doppler flowmetry in assessment of blood flow changes in human masseter muscle induced by isometric contraction. *Arch Oral Biol*. 1989;34:779–786.
21. Curtis DA, Gansky SA, Plesh O. Deep and superficial masseter muscle blood flow in women. *J Prosthodont*. 2012;21:472–477.
22. Jensen BR, Sjøgaard G, Bornmyr S, Arborelius M, Jørgensen K. Intramuscular laser-Doppler flowmetry in the supraspinatus muscle during isometric contractions. *Eur J Appl Physiol Occup Physiol*. 1995;71:373–378.
23. Larsson SE, Zhang Q, Larsson R, Cai H, Oberg PA. Single-fibre laser Doppler flowmetry and electromyography for evaluating microcirculation in forearm muscle during static and continuous handgrip contractions. *Eur J Appl Physiol Occup Physiol*. 1996;73:219–224.
24. Hidaka O, Yanagi M, Takada K. Mental stress-induced physiological changes in the human masseter muscle. *J Dent Res*. 2004;83:227–231.

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