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Publication date
2013
Document Version
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Citation for published version (APA):
Koutris, M. (2013). *Masticatory muscle pain: Causes, consequences, and diagnosis*. [Thesis, fully internal, Universiteit van Amsterdam].

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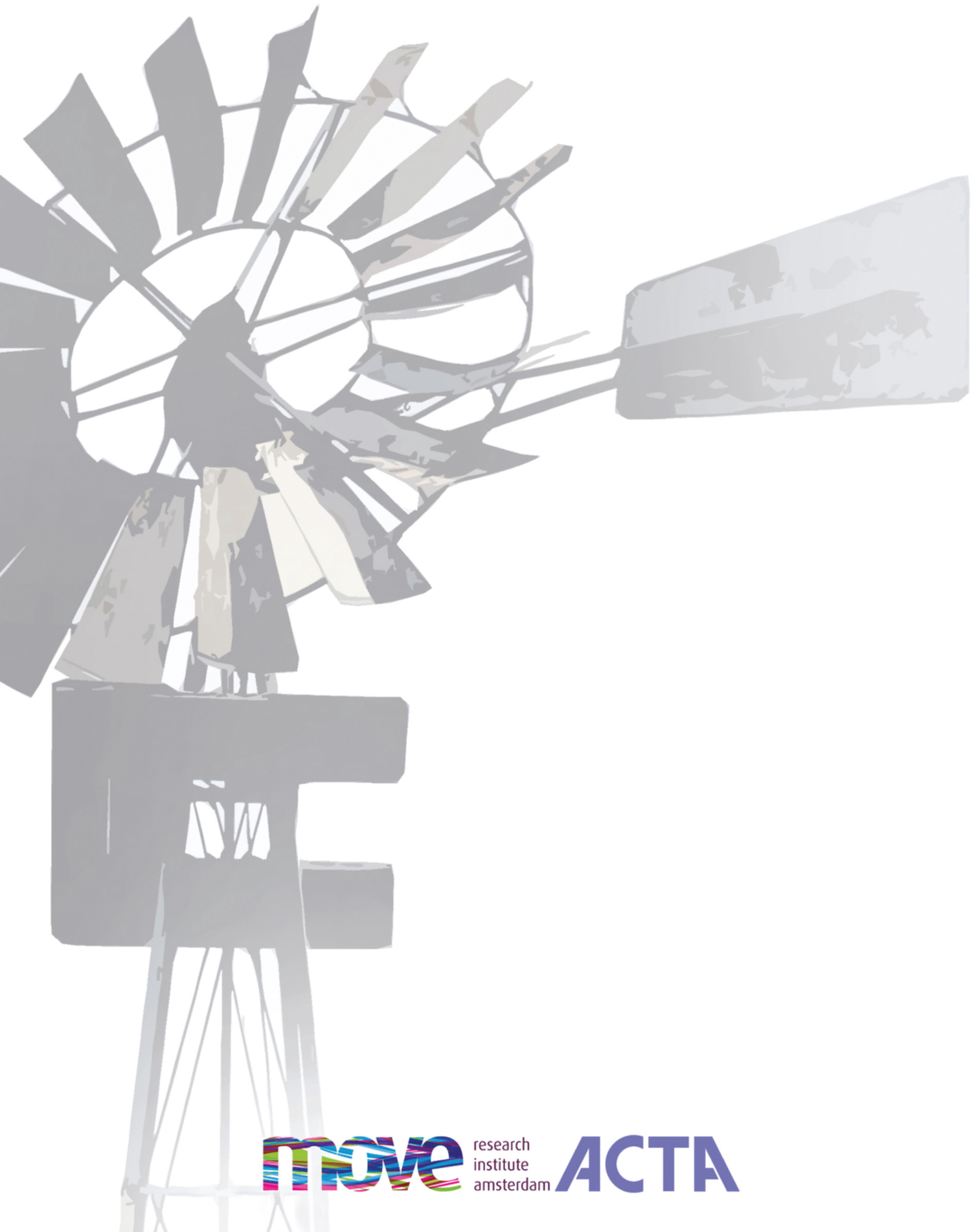

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ACTA

Michail Koutris

Masticatory muscle pain: Causes, consequences, and diagnosis

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This thesis was prepared at the Department of Oral Function and Restorative Dentistry, Section of Oral Kinesiology, of the Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and the Vrije Universiteit, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands.

Supported by the ACTA Research Institute.

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Layout, cover illustration, and design by M. Koutris and L.V. Vassiliou
Printed by:
Print Shop PLETSAS | KARDARI, 107, Har. Trikoupi str., Athens, 11473 | GREECE, email: info@typografio.gr

ISBN/EAN: 978-90-9027831-5

Masticatory muscle pain

Causes, consequences, and diagnosis

ACADEMISCH PROEFSCHRIFT

Ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam

op gezag van de Rector Magnificus

prof. dr. D.C. van den Boom

ten overstaan van een door het college voor promoties

ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op maandag 28 october 2013, te 14:00 uur

door

Michail Koutris

geboren te Rodos, Griekenland

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Faculteit der Tandheelkunde (ACTA)

“Σαν βγεις στον πηγαιμό για την Ιθάκη, να εύχεσαι νάναι μακρύς ο δρόμος, γεμάτος περιπέτειες, γεμάτος γνώσεις.”

Κωνσταντίνος Καβάφης, Ιθάκη

“As you set out for Ithaka hope your road is a long one, full of adventure, full of discovery.”

Constantinos Cavafis, Ithaca

“Als je de tocht aanvaardt naar Ithaka wens dat de weg dan lang mag zijn, vol avonturen, vol ervaringen.”

Constantinos Cavafis, Ithaca

To my Katerina and my little Roula.

To Thomais Zoannou.

To my beloved grandmother, Nina Kountouri.

To my family, Thomas and Evangelia, Thodoris and Katerina Koutris.

To my family in law, Roula Fotopoulou and Aggeliki Tsiliagkou.

To Peter, Miranda, Robert, and Maarten Wetselaar.

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Chapter 1

General Introduction

Musculoskeletal pain in the orofacial region is described under the umbrella term "temporomandibular disorders" (TMD) (McNeil, 1993). Apart from pain arising from the temporomandibular joints (TMJs) and/or the masticatory muscles, TMD also includes internal derangements and degenerative diseases of the TMJs (McNeil, 1993). TMD pain is the most common orofacial pain condition of non-dental origin (de Leeuw, 2008). In the general population, the prevalence of TMD pain is around 10% (LeResche, 1997). Its prevalence is highest in populations aged between 20 and 40 years of age (Drangsholt and LeResche, 1999). Like for other musculoskeletal disorders, TMD pain is more common in women than in men, with a female : male ratio of 2:1 in the general population (Bush et al., 1993). In the population of patients referred to specialized orofacial pain clinics, though, the female : male ratio is considerably higher (up to 9:1) (Bush et al., 1993).

Nowadays, the etiology of TMD-pain is considered multifactorial (Greene, 2001). In this respect, the biopsychosocial model (Engel, 1977;Adler, 2009) is commonly accepted as a framework to describe relevant etiological factors for musculoskeletal pain, including TMD pain. Tissue overloading, like macro- or micro-trauma, are included under the "bio-" component of the model, and are suggested to play an important role in initiating and/or perpetuating musculoskeletal pain (de Leeuw, 2008; Greene, 2001). For the masticatory system, parafunctional activities like tooth grinding and clenching are considered to lead to overloading of the muscle and joint structures. In a systematic review, self-reported parafunctional activities were found to be associated with TMD pain (Manfredini and Lobbezoo, 2010). In a recent report, this association was confirmed (Michelotti et al., 2010). Interestingly, however, when grinding activities were more objectively rated with polysomnographic recordings, the association between parafunctions and TMD pain was no longer found (Raphael et al., 2012). So, the relation between parafunctions and pain is still unclear.

Experimentally induced parafunctions have been studied in order to test whether such activities can indeed initiate pain resembling TMD pain; for a review, see (Svensson et al., 2008). To that end, different kinds of provocation exercises have been developed. In one study, sustained isometric voluntary protrusive jaw-muscle contractions at different clenching levels were not sufficient to provoke pain (Clark et al., 1991). These activities were thought to mimic the clenching component of bruxism. This led to the conclusion that contrary to clinical beliefs, significant jaw pain and tenderness are difficult to induce in healthy participants (Clark et al., 1991). Others tried to mimic long-term awake clenching activity, with results varying from no provocation of pain (Svensson and Arendt-Nielsen, 1996) to provocation of the full spectrum of myofascial TMD-pain complaints (Glaros et al., 1998a; Glaros et al., 1998b; Glaros et al., 2000; Glaros and Burton, 2004). The provoked signs and symptoms of TMD pain, however, were short-lasting and present in a small proportion of the participants only. Other, more dynamic protocols, mimicking tooth grinding movements, provoked significantly elevated pain levels (Arima et al., 1999).

Again, the provoked pain was short lasting and diminished during the days following the provocation part (Arima et al., 1999). To investigate the effect of parafunctional activities on the masticatory system , the study described in **Chapter 2** assesses the effects of experimentally induced jaw-muscle pain by means of intense chewing exercises on the orofacial sensory-motor system. Pain levels (visual analogue scale-VAS scores) and pressure-pain thresholds were measured as sensory components of the masticatory function, while the amplitude of the jaw-stretch reflex was used as motor component of the masticatory function. The results showed that pain levels increased and pressure-pain thresholds diminished after the chewing exercises. However, the motor component of the masticatory function, as quantified by the jaw-stretch reflex amplitude, did not show any change. In a critical reflection on this negative finding, the possibility was considered that the methodology used for the recording of the jaw-stretch reflex was insufficient. More specifically, it was considered whether the amplitude of the jaw-jerk reflex was influenced by spatial differences in day-to-day placement of the electrodes. Therefore, in **Chapter 3,** we studied the spatial dependency of the jaw-stretch reflex recording of the masseter muscle.

As for earlier experimental studies, the symptoms of fatigue and pain provoked by the intense chewing exercise were short lasting, and did not resemble the type of complaints TMD-pain patients present with. Therefore, in **Chapter 4**, a new protocol was developed to induce longer lasting muscle fatigue and pain. This protocol was based on a combination of concentric and eccentric load of the masticatory muscles. Concentric exercises are responsible for short lasting pain of ischemic origin, because they resemble more the usual muscle load during everyday activities. Eccentric muscle contractions, on the other hand, are responsible for delayed and longer lasting pain complaints due to their unaccustomed nature. This type of pain is usually called delayed onset muscle soreness (DOMS) (Proske and Morgan, 2001). In **Chapter 5**, it is investigated whether signs and symptoms of TMD (like subjective feelings of fatigue and pain, limited range of motion, decreased pressure pain thresholds, and muscle tenderness to palpation) can be provoked in healthy individuals with the use of this protocol. It is also examined whether the provoked signs and symptoms mimic those presented by TMD patients.

In clinical practise, for the recognition of pain from the masticatory structures, several diagnostic tests can be used (Lobbezoo-Scholte et al., 1994; Visscher et al., 2000). The most widely used classification system for TMD is the Research Diagnostic Criteria for temporomandibular disorders (RDC/TMD) (Dworkin and LeResche, 1992). It includes two axes: Axis I assesses the physical complaints, while Axis II investigates the psychosocial background of the patient. The clinical examination for an Axis I diagnosis largely depends on standardized palpation of the masticatory muscles. The validity of the RDC/TMD for the recognition of TMD pain is, however, insufficient (Truelove et al., 2010; Visscher et al., 2009). To improve the recognition of TMD-pain, alternative diagnostic tests like

dynamic/static tests have been proposed (Naeije and Hansson, 1986; Visscher et al., 2009). These tests are based on the principle that when a patient's pain complaints arise from the musculoskeletal structures, these complaints should be provoked by functional (dynamic and static) loading of the system (Naeije and Hansson, 1986; Visscher et al., 2009). In addition, the concept of "familiar pain" could improve the validity of clinical tests for TMD-pain (Schiffman et al., 2010; Visscher et al., 2009). When familiar pain is used as the outcome measure of a clinical test, only a response of pain that resembles the patient's current complaints is considered positive. Finally, the validity of clinical test could be confounded by the presence of comorbid factors, like widespread pain or depression. Even though the negative influence of comorbidity on the prognosis of TMD-pain treatment is well-known (Hoffmann et al., 2011), its influence on the validity of clinical tests for musculoskeletal pain has not been studied before. Therefore, in **Chapter 6**, the influence of comorbidity on diagnostic tests for TMD is studied, together with the role of the addition of the familiar pain concept in in the diagnostic accuracy of both palpation and dynamic/static tests.

REFERENCES

Adler RH (2009). Engel's biopsychosocial model is still relevant today. *J Psychosom Res* 67(6)*:*607-611.

Arima T, Svensson P, Arendt-Nielsen L (1999). Experimental grinding in healthy subjects: a model for postexercise jaw muscle soreness? *J Orofac Pain* 13(2)*:*104-114.

Bush FM, Harkins SW, Harrington WG, Price DD (1993). Analysis of gender effects on pain perception and symptom presentation in temporomandibular pain. *Pain* 53(1)*:*73-80.

Clark GT, Adler RC, Lee JJ (1991). Jaw pain and tenderness levels during and after repeated sustained maximum voluntary protrusion. *Pain* 45(1)*:*17-22.

de Leeuw R (2008). Orofacial Pain. Guidelines for assessment, diagnosis, and management. Chicago: Quintessence Publishing Co, Inc.

Drangsholt M, LeResche L (1999). Temporomandibular Disorder Pain. In: Epidemiology of Pain. IK Crombie editor. Seattle: IASP Press.

Engel GL (1977). The need for a new medical model: a challenge for biomedicine. *Science* 196(4286)*:*129-136.

Glaros AG, Baharloo L, Glass EG (1998a). Effect of parafunctional clenching and estrogen on temporomandibular disorder pain. *Cranio* 16(2)*:*78-83.

Glaros AG, Tabacchi KN, Glass EG (1998b). Effect of parafunctional clenching on TMD pain. *J Orofac Pain* 12(2)*:*145-152.

Glaros AG, Forbes M, Shanker J, Glass EG (2000). Effect of parafunctional clenching on temporomandibular disorder pain and proprioceptive awareness. *Cranio* 18(3)*:*198-204.

Glaros AG, Burton E (2004). Parafunctional clenching, pain, and effort in temporomandibular disorders. *J Behav Med* 27(1)*:*91-100.

Greene CS (2001). The etiology of temporomandibular disorders: implications for treatment. *J Orofac Pain* 15(2)*:*93-105; discussion 106-116.

Hoffmann RG, Kotchen JM, Kotchen TA, Cowley T, Dasgupta M, Cowley AW, Jr. (2011). Temporomandibular disorders and associated clinical comorbidities. *Clin J Pain* 27(3)*:*268-274.

LeResche L (1997). Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med* 8(3)*:*291-305.

Lobbezoo-Scholte AM, de Wijer A, Steenks MH, Bosman F (1994). Interexaminer reliability of six orthopaedic tests in diagnostic subgroups of craniomandibular disorders. *J Oral Rehabil* 21(3)*:*273-285.

Manfredini D, Lobbezoo F (2010). Relationship between bruxism and temporomandibular disorders: a systematic review of literature from 1998 to 2008. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 109(6)*:*e26-50.

McNeil C (1993). Temporomandibular Disorders. Second ed. Illinois: Quintessence Publishing Co.

Michelotti A, Cioffi I, Festa P, Scala G, Farella M (2010). Oral parafunctions as risk factors for diagnostic TMD subgroups. *J Oral Rehabil* 37(3)*:*157-162.

Naeije M, Hansson TL (1986). Electromyographic screening of myogenous and arthrogenous TMJ dysfunction patients. *J Oral Rehabil* 13(5)*:*433-441.

Proske U, Morgan DL (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 537(Pt 2)*:*333-345.

Raphael KG, Sirois DA, Janal MN, Wigren PE, Dubrovsky B, Nemelivsky LV *et al.* (2012). Sleep bruxism and myofascial temporomandibular disorders: A laboratory-based polysomnographic investigation. *J Am Dent Assoc* 143(11)*:*1223-1231.

Schiffman EL, Truelove EL, Ohrbach R, Anderson GC, John MT, List T *et al.* (2010). The Research Diagnostic Criteria for Temporomandibular Disorders. I: overview and methodology for assessment of validity. *J Orofac Pain* 24(1)*:*7-24.

Svensson P, Jadidi F, Arima T, Baad-Hansen L, Sessle BJ (2008). Relationships between craniofacial pain and bruxism. *J Oral Rehabil* 35(7)*:*524-547.

Truelove E, Pan W, Look JO, Mancl LA, Ohrbach RK, Velly AM *et al.* (2010). The Research Diagnostic Criteria for Temporomandibular Disorders. III: validity of Axis I diagnoses. *J Orofac Pain* 24(1)*:*35-47.

Visscher CM, Lobbezoo F, de Boer W, van der Zaag J, Verheij JG, Naeije M (2000). Clinical tests in distinguishing between persons with or without craniomandibular or cervical spinal pain complaints. *Eur J Oral Sci* 108(6)*:*475-483.

Visscher CM, Naeije M, De Laat A, Michelotti A, Nilner M, Craane B *et al.* (2009). Diagnostic accuracy of temporomandibular disorder pain tests: a multicenter study. *J Orofac Pain* 23(2)*:*108-114.

Chapter 2

Effects of intense chewing exercises on the masticatory sensory-motor system

Koutris M, Lobbezoo F, Naeije M, Wang K, Svensson P, Arendt-Nielsen L, Farina D

J Dent Res 2009; 22(4):658-662

ABSTRACT

Nociceptive substances, injected into the masseter muscle, induce pain and facilitate the jaw-stretch reflex. It is hypothesized that intense chewing would provoke similar effects. Fourteen men performed 20 bouts of 5-minutes chewing. After each bout, 20 minutes and 24 hrs after the exercise, muscle fatigue and pain scores and the normalized reflex amplitude from the left masseter muscle were recorded. Before, 20 min, and 24 hrs after the exercise, signs of temporomandibular disorders and pressure pain thresholds of the masticatory muscles were also recorded. Fatigue and pain scores had increased during the exercise ($P<0.001$), but the reflex amplitude did not ($P=0.123$). Twenty minutes after the exercises, 12 participants showed signs of myofascial pain or arthralgia. Pressure-pain thresholds were decreased after 20 min ($P=0.009$) and 24 hrs ($P=0.049$). Intense chewing can induce fatigue, pain, and decreased pressure-pain thresholds in the masticatory muscles, without concomitant changes in the jaw-stretch reflex.

Key words: jaw-stretch reflex, chewing exercises, fatigue, pain

ACKNOWLEDGMENTS

This research was supported by the European Community “Marie Curie Training Sites” program (HPMT-CT-2000-00092).

INTRODUCTION

Chewing is an oral function that is of vital importance for the biological and social life of human beings. As a prerequisite for this function, a healthy masticatory system, characterized by the absence of pain at rest and during functional movements of the mandible, is required. Pain, in contrast, may influence the characteristics of the masticatory sensory-motor system (Svensson and Graven-Nielsen, 2001, Lobbezoo *et al.*, 2006).

Study to the jaw-stretch reflex can provide insight into the characteristics of the masticatory motor system (i.e., sensitivity of the motor neurons and muscle spindles). Studies have demonstrated that nociceptive substances such as hypertonic saline, glutamate, and capsaicin, injected into the masticatory muscles, can modulate the amplitude of this reflex (Wang *et al.*, 2000, 2002, 2004; Svensson *et al.*, 2001a, 2003; Cairns *et al.*, 2003; van Selms *et al.*, 2005). However, the characteristics of muscle pain, reported by persons with myofascial temporomandibular disorders (TMD), are probably more similar to those of exercise-induced muscle pain than to those of acute, short lasting pain evoked by exogenous pain provocation techniques (Stohler, 1999; Svensson and Graven-Nielsen, 2001). Therefore, the aim of this study was to investigate the effects of exercise-induced muscle fatigue and pain upon sensory-motor aspects of the masticatory system. Sensory aspects were assessed by analysis of the visual analog scale (VAS) scores for muscle pain and fatigue, by determination of the muscles' pressure-pain threshold, and by assessment of the muscle pain according to the research diagnostic criteria for TMD (RDC/TMD). Motor aspects were assessed by analysis of the amplitude of the jaw-stretch reflex.

MATERIALS & METHODS

Participants

Fourteen men (age, mean ± SD = 24.4 ± 2.8 years), free of dental pathologies, or TMDs (Dworkin and LeResche, 1992), participated in the study. Individuals, who chewed gum regularly (i.e., more than 30 min a day), or who reported sleep or wake bruxism were excluded. Before the start of the experiment, participants signed a written informed consent. The study was conducted in accordance with the Helsinki Declaration and was approved by the local Ethics Committee (20040074).

Study design

Participants underwent 20 consecutive bouts of 5-minute chewing exercise. During each chewing bout, subjects chewed on 7 pieces (7 x 1.4 gr = 9.8 gr) of hard chewing gum (ELMA®, Mastiha, Chios, Greece), at a speed of 1.5 times their individual chewing pace (van Selms *et al.*, 2005). After each chewing bout, the VAS scores of fatigue and pain intensity, and the normalized peak-to-peak amplitude of the jaw-stretch reflex were recorded. The same data were also collected 20 min and 24 hrs after the end of the last chewing bout. Before the start of the experiment, and 20 min and 24 hrs afterwards, participants also underwent a clinical examination according to the RDC/TMD, and

pressure pain thresholds of the left (LM) and right (RM) masseters and the left (LAT) and right (RAT) anterior temporalis muscles were determined.

Fatigue and pain intensity

Fatigue and pain intensity in the jaw muscles were recorded on two, 100-mm VASs, with left anchor words "No fatigue at all" and "No pain at all", and right anchor words "Fatigue as worse as it could be" and "Pain as worse as it could be", respectively.

Jaw-stretch reflex recordings

Jaw-stretch reflexes were evoked by jaw displacements of 1 mm with a ramp time of 10 ms, using a muscle stretcher as described previously (Miles *et al.,* 1993). The initial jaw gape, determined by the distance between the upper and lower jaw bars, was 4 mm.

Electromyographic activity of the left masseter muscle was recorded with bipolar surface electrodes (4 x 7 mm recording area, 720-01-k, Neuroline, Medicotest, Ølstykke, Denmark). Electrodes were placed 10 mm apart, parallel to the main direction of the muscles fibres over the palpated belly of the muscle, after skin preparation with alcohol. A ground electrode, soaked with saline, was attached to the right wrist. To ensure that the electrodes were placed accurately at the same sites at the 24-hour-recovery appointment, we made a lateral drawing of the head on transparent plastic foil. The electromyographic signal was amplified (200 to 5000 times), filtered (bandwidth 20 Hz to 1 kHz), sampled (4 kHz), and stored for offline analysis.

During the recordings of the reflexes, the subjects were seated upright in front of the muscle stretcher, while biting with their incisors on 2 jaw bars. They contracted their muscles at a clenching level of about 15% of the pre-fatigued maximum electromyographic activity of the left masseter muscle. The pre-fatigued maximum activity was determined before the start of the exercises, by three, 5-second maximal clenches performed with the central incisors on the bars of the jaw-stretcher. The mean electromyographic value of the three clenches was taken as the pre-fatigued maximum electromyographic activity (van Selms *et al.*, 2005).

After each chewing bout, 20 jaw-stretch reflexes were evoked. For each stimulus, 300 ms of peri-stimulus electromyographic activity was recorded, with 100 ms pre-stimulus and 200 ms post-stimulus. The 100 ms electromyographic signal preceding each stimulus was full-wave rectified, and its averaged value was calculated. The mean of these 20 average electromyographic values was considered the mean pre-stimulus electromyographic activity. The electromyographic signals following each stimulus were averaged over the 20 sweeps and the jaw-stretch reflex appeared as a biphasic potential in this average sweep. The amplitude of the reflex was the peak-to-peak value of this biphasic potential. Subsequently, the reflex amplitude was normalized with respect to the mean pre-stimulus electromyographic activity.

Pressure-Pain Threshold values

Pressure-pain threshold is defined as the amount of pressure (kPa) which the participants first perceived as painful (Svensson *et al.*, 1995). We used an electronic pressure algometer with an algometer probe diameter of 1 cm (Somedic AB, Farsta, Sweden) to assess the muscle's pressure-pain threshold. At each occasion, four pressure pain threshold recordings *per* muscle were made at a constant application rate of 30 kPa/s. The mean values of the last 3 recordings were used in the subsequent analysis (Isselee *et al.*, 1998). For determination of the site for the pressure application, participants were asked to clench and relax several times, and the area of greatest bulge of the muscle was marked on the same plastic transparent foil used for the electrodes.

Statistical analysis

During the chewing exercise, changes in the VAS scores of muscle fatigue and pain, and in the normalized jaw-stretch reflex amplitude, were analyzed by the cumulative sum (CUSUM) technique (Ellaway, 1978). CUSUM is calculated according to the equation:

$$CUSUM_i = \sum_{j=1}^{j=20} (VAR_{i,j} - k_i)$$

where the subscript '*i*' denotes subject '*i*', k_i indicates the pre-exercise value of the variable '*VAR*', and '$VAR_{i,j}$' corresponds to the variable value of subject '*i*' at time '*j*'. One-sample t-tests assessed whether the CUSUM values immediately after the chewing exercise differed from zero.

The VAS scores and reflex data collected before, and 20 min and 24 hrs after the exercise were analyzed with ANOVA for repeated measures, with time as a within-subjects factor with 3 levels (Before, After 20 min, After 24 hrs). Pressure-pain threshold values were also analyzed by ANOVA for repeated measures, with as additional within-participant factor "muscle", consisting of 4 levels (LM, RM, LAT, and RAT). When sphericity was violated, conservative Greenhouse-Geisser corrected values of the degree of freedom were used (Mauchly, 1940; Greenhouse and Geisser, 1959). Pairwise comparisons were performed using Dunn-Sidak *post hoc* correction for multiple comparisons.

The level of statistically significant difference was set at $\alpha = 0.05$. All analyses were performed with SPSS software, version 12.0.1.

Table 1. Individual Cumulative Sum (CUSUM) values at the end of the chewing exercise for the VAS scores of fatigue and pain, and for the normalized reflex amplitude. (the p-values indicate whether the CUSUM values were significantly different from zero) (N=14)

	Cumulative Sum (CUSUM) values		
Participant No	VAS Scores of Fatigue	VAS Scores of Pain	Normalized reflex amplitude
1	804	466	-5.6
2	1342	240	-10.1
3	553	420	135.3
4	334	0	-126.8
5	1188	189	76.2
6	60	5	195.6
7	596	4	101.6
8	1234	772	-17.2
9	1088	0	-79.2
10	674	573	-6.9
11	245	36	153.9
12	658	375	30
13	1029	392	26.4
14	1382	588	47.9
Mean value ± SD	799 ± 422.3 (P < 0.001)	290 ± 259.5 (P < 0.001)	39.3 ± 89.4 (P = 0.123)

Table 2. Mean value and standard deviation of the visual analogue scales (VAS) scores of fatigue and pain, and of the normalized reflex amplitude, recorded before the start of the chewing exercises (Before), 20 min after the end of chewing (After 20 min) and 24 hrs after the end of chewing (After 24 hrs). (N=13)

	Before	After 20 Min	After 24 Hrs	ANOVA
VAS scores of fatigue	1.4 ± 2.3	26.8 ± 22.5	4.8 ± 9.5	F = 11.0; P = 0.004
VAS scores of pain	1 ± 2.3	4.9 ± 8.9	0.3 ± 1.1	F = 3.2; P = 0.1
Normalized reflex amplitude	19.9 ± 11.3	21.8 ± 13	19.3 ± 10	F = 0.6; P = 0.5

RESULTS

Twelve participants completed the entire exercise of 20 chewing bouts. One participant was already exhausted after the 16th chewing bout, and one participant after the 18th chewing bout. One person did not participate in the 24-hours-recovery measurements, and his data were excluded from the analysis of the recovery data.

Provocation

The mean chewing pace for the participants was 106 cycles/min. During the chewing exercise, the mean VAS scores for fatigue and pain gradually increased. Their CUSUM values at the end of the exercise were significantly different from zero ($P<0.001$) (Fig. 1, Table 1). The normalized reflex amplitudes showed no tendency to change during the chewing exercise (Table 1, $P=0.123$).

Recovery

ANOVA for repeated measures showed a significant effect of the factor time on the VAS scores of fatigue, but not on the VAS scores of pain or on the normalized reflex amplitude (Table 2). *Post hoc* analysis showed that 20 min after the last chewing bout, the VAS scores of fatigue were significantly different from those before the start of the experiment ($P = 0.004$), while after 24 hrs, no difference was found ($P = 0.5$).

The pressure-pain threshold values showed no difference between the muscles studied ($F = 1.077$, $P = 0.354$), but there was a significant influence of the factor time ($F = 7.934$, $P = 0.002$). *Post hoc* analysis showed significant differences between the baseline pressure pain threshold values and the values 20 min ($P = 0.009$) and 24 hrs ($P = 0.049$) after the exercise (Fig. 2).

The RDC/TMD examination, performed after the chewing exercise, revealed the clinical characteristics of a myofascial pain without limited mouth opening in 11 participants; one of them still had these characteristics 24 hrs later. The characteristics of myofascial pain with limited mouth-opening and of arthralgia in both the temporomandibular joints were observed in another participant, also 24 hrs after the exercise. The two participants who reported pain after 24 hrs were also contacted one day later, and reported no longer experiencing pain or fatigue.

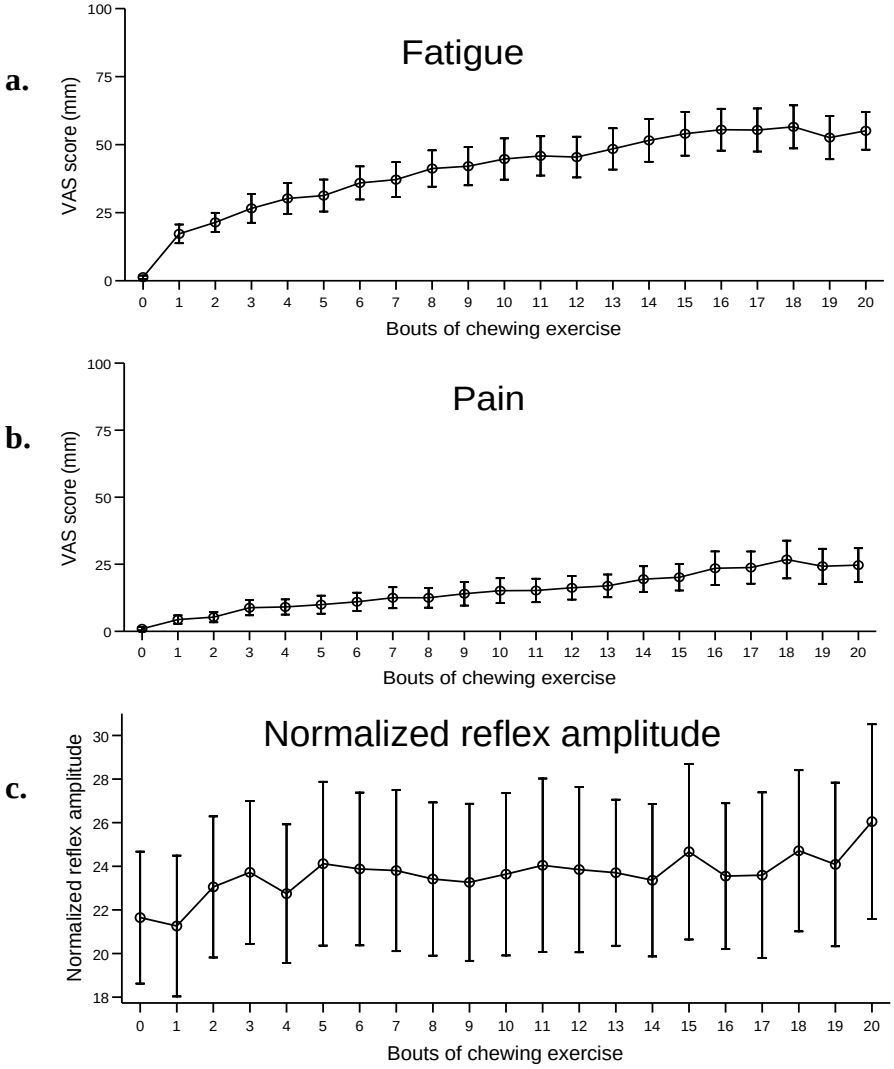

Figure 1. Mean value and standard deviation of the fatigue visual analogue scales (VAS) scores (**a**), the pain VAS scores (**b**) and the normalized jaw-stretch reflex amplitude (**c**), recorded before and during the protocol of 20 bouts of chewing (n=14).

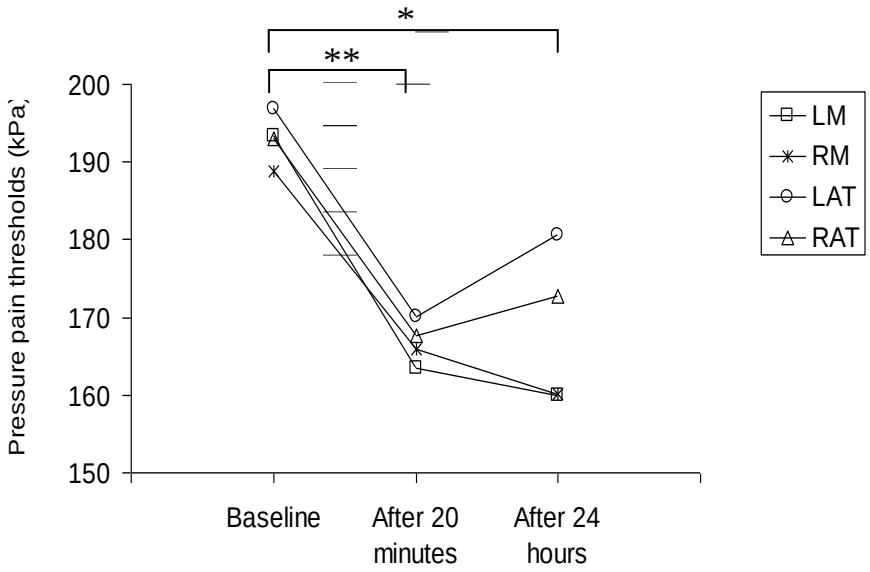

Figure 2. Pressure pain threshold values of the left masseter (LM), right masseter (RM), left anterior temporalis (LAT) and right anterior temporalis (RAT), recorded before, 20 min after, and 24 hrs after the chewing experiment (*: $P < 0.05$, **: $P < 0.01$) (N=13).

DISCUSSION

In this study, the effects of exercise-induced muscle fatigue and pain upon sensory-motor aspects of the masticatory system were analyzed. A heavy chewing protocol resulted in short-lasting muscle fatigue and pain and in a lower muscle pressure pain threshold, but not in a change in the amplitude of the jaw-stretch reflex.

Women show a tendency to report significantly elevated levels of muscle pain after a 6-minute chewing exercise, while men do not (Karibe *et al.*, 2003). Therefore, to exclude possible gender differences in the reports of muscle pain and fatigue, we selected participants of only one gender (males) in this study. Participants with self-reports of bruxism and with reports of gum chewing for more than 30 min on a daily basis, were also excluded, since these activities may influence the resistance to fatigability of the chewing muscles (Cheung *et al.*, 2003).

That a heavy chewing exercise can provoke fatigue and pain in the masticatory muscles has been reported before (Bakke *et al.*, 1996; Farella *et al.*, 1999, 2001). However, this study has shown that such a chewing exercise can also lead to signs and symptoms which are characteristic of myofascial pain and of arthralgia, as defined by the RDC/TMD. In two participants, these signs and symptoms were still present 24 hrs after the exercise.

This may underline the suggestion that signs and symptoms of TMD, as recognized by the RDC/TMD, may be associated with masticatory muscle hyperactivity (Okeson, 1996; Svensson and Graven-Nielsen, 2001).

It is likely that the pain experienced during and immediately after the exercise was caused by ischemia of the masticatory muscles and the subsequent accumulation of metabolic products in these muscles (Stohler, 1999; Svensson *et al.,* 2001b, Nicol et al., 2003). This would also explain why the feelings of fatigue and pain had disappeared so soon after the exercise; blood flow through the masticatory muscles is restored quickly after the cessation of exercise (Inoue-Minakuchi *et al.,* 2001). Despite the fact, that the pain and fatigue VAS scores after 24 hrs were not statistically different from their baseline values, the muscles' pressure-pain threshold values were still lower at that time. This indicates that participants whose muscles were pain-free after the exercise, may nevertheless show an increased sensitivity to palpation of these muscles. This stresses the need for further research into the role of palpation tests in the recognition of musculoskeletal disorders such as TMD (Dworkin and LeResche, 1992).

In this study, large differences in the response of the jaw-stretch reflex amplitude to the chewing exercise were found between participants, despite serious efforts to standardize the study protocol and despite the normalization of the reflex amplitude. Under the present experimental conditions and at a group level, the normalized reflex amplitude remained unchanged during the chewing exercise. These findings corroborate the results of a previous study in which fatigue induced by 6 min of chewing did not modulate the normalized reflex amplitude (van Selms *et al.*, 2005), but are in contrast to studies where pain, induced by injection of various substances into the masseter muscle, facilitated the jaw-stretch reflex. However, the injection of hypertonic saline induces pain of around 50 mm on a 100 mm VAS (Svensson *et al.*, 1995, van Selms *et al.*, 2005), while our chewing exercise resulted in lower VAS levels of around 25 mm. Even though there is discussion in the literature regarding the linear (Hofmans and Theuns, 2008) or non-linear (Pesudovs *et al.*, 2005) behaviour of the VAS, these results do indicate that the injection of hypertonic saline induces more pain in the muscles than does chewing exercise. These differences in the intensity of pain provoked by the two methods may account for the differences in the modulation of the jaw-stretch reflex. It also raises the question whether the jaw-stretch reflex changes, provoked by substance-induced muscle pain, will ever occur during 'everyday life' exercise-induced muscle pain.

Differences in the way exercise-induced muscle fatigue and/or pain influence the stretch reflex have also been observed in other muscles of the human body. Some studies showed an inhibition of the stretch reflex following exhaustive exercise (Nicol *et al.*, 1996, 2003, Regueme *et al.*, 2005), others showed no influence (Hjortskov *et al.*, 2005), while moderate muscle fatigue has been reported to cause a slight facilitation of the stretch reflex (Komi, 2000). Unfortunately, differences in the muscle pain and fatigue inducing protocols

and in the skeletal muscles under study make it impossible to draw definite conclusions at this stage.

Within the limitations of this study, it can be concluded that intense and prolonged chewing exercise can induce fatigue, pain, and a decreased pressure pain threshold in the human masticatory muscles, without concomitant changes in the jaw-stretch reflex amplitude.

REFERENCES

American Academy of Orofacial Pain. (1988). Orofacial Pain. Guidelines for Assessment, Diagnosis, and Management. Okeson JP, editor. Chicago: Quintessence p. 120.

Bakke M, Thomsen CE, Vilmann A, Soneda K, Farella M, Moller E (1996). Ultrasonographic assessment of the swelling of the human masseter muscle after static and dynamic activity. *Arch Oral Biol* 41:133-140.

Cairns BE, Wang K, Hu JW, Sessle BJ, Arendt-Nielsen L, Svensson P (2003). The effect of glutamate-evoked masseter muscle pain on the human jaw-stretch reflex differs in men and women. *J Orofac Pain* 17:317-325.

Cheung K, Hume P, Maxwell L (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 33:145-164.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6:301-355.

Ellaway PH (1978). Cumulative sum technique and its application to the analysis of peristimulus time histogram. *Electroenceph Clin Neurophysiol* 45:302-304.

Farella M, Bakke M, Michelotti A, Marotta G, Martina R (1999). Cardiovascular responses in humans to experimental chewing of gums of different consistencies. *Arch Oral Biol* 44:835-842.

Farella M, Bakke M, Michelotti A, Martina R (2001). Effects of prolonged gum chewing on pain and fatigue in human jaw muscles. *Eur J Oral Sci* 109:81-85.

Greenhouse SW, Geisser S (1959). On methods in the analysis of profile data, *Psychometrika* 24:95–111.

Hjortskov N, Essendrop M, Skotte J, Fallentin N (2005). The effect of delayed-onset muscle soreness on stretch reflexes in human low back muscles. *Scand J Med Sci Sports* 15:409-415.

Hofman J, Theuns P (2008). On the linearity of predefined and self-anchoring Visual Analogue Scales. *Br J Math Stat Psychol* 61(Pt2):401-413.

Inoue-Minakuchi M, Maekawa K, Kuboki T, Suzuki K, Yamashita A, Yatani H, *et al*. (2001). Intramuscular haemodynamic responses to different durations of sustained extension in normal human masseter. *Arch Oral Biol* 46:661-666.

Isselee H, De Laat A, Bogaerts K, Lysens R (1998). Short-term reproducibility of pressure pain thresholds in masticatory muscles measured with a new algometer. *J Orofac Pain* 12:203-209.

Karibe H, Goddard G, Gear RW (2003) Sex differences in masticatory muscle pain after chewing. *J Dent Res* 82:112-116.

Komi PV (2000). Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. *J Biomech* 33:1197-1206.

Lobbezoo F, van Selms MK, Naeije M (2006). Masticatory muscle pain and disordered jaw motor behaviour: literature review over the past decade. *Arch Oral Biol* 51:713-720.

Mauchly JW (1940). Significance test for sphericity of a normal *n*-variate distribution. *Ann Math Stat* 11:204–209.

Miles TS, Poliakov AV, Flavel SC (1993). An apparatus for controlled stretch of human jaw-closing muscles. *J Neurosci Methods* 46:197-202.

Nicol C, Komi PV, Horita T, Kyrolainen H, Takala TE (1996). Reduced stretch-reflex sensitivity after exhausting stretch-shortening cycle exercise. *Eur J Appl Physiol Occup Physiol* 72:401-409.

Nicol C, Kuitunen S, Kyrolainen H, Avela J, Komi PV (2003). Effects of long- and short-term fatiguing stretch-shortening cycle exercises on reflex EMG and force of the tendon-muscle complex. *Eur J Appl Physiol* 90:470-479.

Pesudovs K, Craigie MJ, Roberton G (2005). The visual analogue scale for the measurement of pain is not linear. *Anaesth Intensive Care* 33:686-687.

Regueme SC, Nicol C, Barthelemy J, Grelot L (2005). Acute and delayed neuromuscular adjustments of the triceps surae muscle group to exhaustive stretch-shortening cycle fatigue. *Eur J Appl Physiol* 93:398-410.

Stohler CS (1999). Craniofacial pain and motor function: pathogenesis, clinical correlates, and implications. *Crit Rev Oral Biol Med* 10:504-518.

Svensson P, Arendt-Nielsen L, Nielsen H, Larsen JK (1995). Effect of chronic and experimental jaw muscle pain on pain-pressure thresholds and stimulus-response curves. *J Orofac Pain* 9:347-356.

Svensson P, Graven-Nielsen T (2001). Craniofacial muscle pain: review of mechanisms and clinical manifestations. *J Orofac Pain* 15:117-145.

Svensson P, Macaluso GM, De Laat A, Wang K (2001a). Effects of local and remote muscle pain on human jaw reflexes evoked by fast stretches at different clenching levels. *Exp Brain Res* 139:495-502.

Svensson P, Burgaard A, Scholler S (2001b). Fatigue and pain in human jaw muscles during a sustained, low-intensity clenching task. *Arch Oral Biol* 46:773-777.

Svensson P, Wang K, Arendt-Nielsen L (2003). Effect of muscle relaxants on experimental jaw-muscle pain and jaw-stretch reflexes: a double-blind and placebo-controlled trial. *Eur J Pain* 7:449-456.

van Selms MK, Wang K, Lobbezoo F, Svensson P, Arendt-Nielsen L, Naeije M (2005). Effects of masticatory muscle fatigue without and with experimental pain on jaw-stretch reflexes in healthy men and women. *Clin Neurophysiol* 116:1415-1423.

Wang K, Svensson P, Arendt-Nielsen L (2000). Effect of tonic muscle pain on short-latency jaw-stretch reflexes in humans. *Pain* 88:189-197.

Wang K, Arendt-Nielsen L, Svensson P (2002). Capsaicin-induced muscle pain alters the excitability of the human jaw-stretch reflex. *J Dent Res* 81:650-654.

Wang K, Sessle BJ, Svensson P, Arendt-Nielsen L (2004). Glutamate evoked neck and jaw muscle pain facilitate the human jaw stretch reflex. *Clin Neurophysiol* 115:1288-1295.

Chapter 3

Normalization reduces the spatial dependency of the jaw-stretch reflex activity in the human masseter muscle

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Muscle Nerve 2010; 41(1):78-84

ABSTRACT

The jaw-stretch reflex is the short-latency response in the jaw-closing muscles after a sudden stretch. The hypothesis whether normalization of the jaw-stretch reflex amplitude with respect to prestimulus electromyographic (EMG) activity will make the amplitude more independent of the location of the electrodes over the masseter muscle was tested. A 5 × 6 electrode grid was used to record the jaw-stretch reflex from 25 sites over the right masseter muscle of 15 healthy men. The results showed that there was a significant site dependency of the prestimulus EMG activity and the reflex amplitude. High cross-correlation coefficients were found between the spatial distribution of mean prestimulus EMG activities and reflex amplitude. When the reflex amplitude was normalized with respect to the prestimulus EMG activity, no site dependency was found. In conclusion, normalization of the jaw-stretch reflex amplitude by the pre-stimulus EMG activity strongly reduces its spatial dependency.

Key words: Jaw-stretch reflex, multichannel EMG, normalization, human masseter muscle

ACKNOWLEDGMENTS

Supported by a Marie Curie Fellowship of the European Community program "Marie Curie Training Sites" under contract number HPMT-CT-2000-00092 and by the Danish Technical Research Council (project "Centre for Neuroengineering (CEN)," contract number 26-04-0100) (to D.F.). The authors are sincerely grateful to Fredéric Leclerc (Université d' Orléans, France) for technical support during the experimental and data analysis.

INTRODUCTION

The jaw-stretch reflex is the short-latency excitatory response in the jaw-closing muscles after the application of a sudden stretch. It is considered the trigeminal equivalent of the monosynaptic spinal stretch reflex in limb muscles (Lund et al., 1983). The reflex is important in the maintenance of the posture of the mandible against the gravitational load during running (Miles, 2007), for compensating sudden changes in load during chewing, and for development of powerful bite forces in aggressive or defensive situations (Scutter and Türker, 2001). The simplest way to provoke the jaw-stretch reflex is by tapping the chin with a reflex hammer (Murray and Klineberg, 1984). However, this method does not allow the control of important parameters such as the amount of displacement of the mandible or the applied force. More complex systems that control the displacement of the mandible allow the reflex provocation under standardized conditions (Lobbezoo et al., 1993a; Miles et al., 1993; Svensson et al., 2000).

The jaw-stretch reflex can be recorded from the jaw-closing muscles with the use of bipolar surface electrodes (Wang and Svensson, 2001). It appears as a biphasic excitatory potential in the raw electromyographic (EMG) signal (Wang et al., 2000). The voluntary EMG activity has been reported to vary between different recording sites over the masseter muscle (Schumann et al., 1994; Castroflorio et al., 2005) but little is known about the spatial dependency of the amplitude of the jaw-stretch reflex. If the reflex amplitude follows the same spatial distribution as the voluntary EMG activity, it may be expected that normalizing the amplitude with respect to the voluntary activity preceding the reflex stimulus, could reduce the dependency on the recording site.

The aim of the study was to test the hypothesis that normalization of the jaw-stretch reflex amplitude with respect to the voluntary EMG activity preceding the reflex stimulus makes the amplitude more independent of the electrode location over the masseter muscle.

MATERIALS AND METHODS

Participants

Fifteen men (mean age ± SD = 24.6 ± 2.1 years) who were free of temporomandibular disorders (Dworkin and LeResche, 1992) participated in the study. All had full, healthy dentition (≥28 teeth), without periodontal or endodontic problems, or extensive restorative works. Before the start of the experiment, the participants were given detailed information about the experimental procedure, and when they agreed to proceed, they signed a written informed consent for their voluntary participation. The experiment was conducted in accordance to the Helsinki Declaration, and the protocol was approved by the local Ethics Committee of the University of Aalborg (VN20040074).

Elicitation of the Jaw-Stretch Reflex

The jaw-stretch reflexes were evoked with a custom-built muscle stretcher based on the original design of Miles (Miles et al., 1993). Briefly, it consisted of two stainless-steel jaw bite-bars which were 1.5 cm wide and 2 cm long each. The upper jaw bite-bar was mounted on a frame firmly attached to the floor. A powerful electromagnetic vibrator (Ling Dynamic Systems, model 406, UK) imposed servo-controlled displacements of the lower jaw bite-bar. A 200 N load cell (Kistler 5039 A312, Switzerland), placed in series with the moveable probe of the vibrator, measured forces on the lower jaw bar. The displacement of the vibrator probe was measured with a linear potentiometer (Sakae Type 20FLP 30A-5K, Japan) mounted parallel to the vibrator probe. Acceleration in the vertical plane was measured with an accelerometer (Delta Tron Accelerometer Type 4399, Brüel and Kjær, Denmark) mounted on the lower jaw bar. The jaw-stretch reflex was evoked by a displacement of the lower bar of 1 mm with a ramp time of 10 ms. The initial jaw gape, determined by the distance between the outer surfaces of the upper and lower jaw bars, was 4 mm (Poliakov and Miles, 1994).

Surface EMG recordings

A bipolar surface EMG signal was recorded from the left masseter muscle (electrodes 720-01-k, 4 x 7 mm recording area, Neuroline, Medicotest, Ølstykke, Denmark). The electrodes were placed 10 mm apart over the muscle belly (Fig. 1) after skin preparation with alcohol. The bipolar EMG signal was amplified (200 - 5000 times; Counterpoint MK2, Skovlunde, Denmark), filtered (bandwidth 20 Hz – 1 kHz), and sampled at 4 kHz.

Multi-channel bipolar surface EMG recordings were obtained from the right masseter muscle using a 5 × 6 electrode grid (LISiN-OT Bioelettronica, Torino, Italy). The electrode diameter was 1 mm, and there was a distance of 8 mm between electrodes in both the posterior-anterior and caudal-cranial directions. For each column of electrodes, multi-channel bipolar recordings were obtained from adjacent electrodes in the caudal-cranial direction (Fig. 1). This analysis provided a total of 25 bipolar recordings. To locate the grid, the inferior borders of the mandible and the zygomatic arch, together with the posterior border of the mandibular ramus were marked on the skin using a skin-marking pen. The anterior border of the masseter was identified by palpation during subsequent clenching and relaxing tasks. After the skin was prepared using abrasive paste, the electrode grid was mounted with the first anterior column of electrodes corresponding to the anterior border of the masseter and the last row corresponding to the inferior border of the mandible (Fig. 1). The multi-channel bipolar EMG signals were amplified (64-channel surface EMG amplifier, SEA 64, OT Bioelectronica, Torino, Italy; -3dB bandwidth 10 - 500 Hz) with a gain of 2000 - 5000 and sampled at 2048 Hz with 12-bit resolution. A ground electrode was placed around the right wrist to serve as common reference for the EMG signals recorded from the left and right masseters.

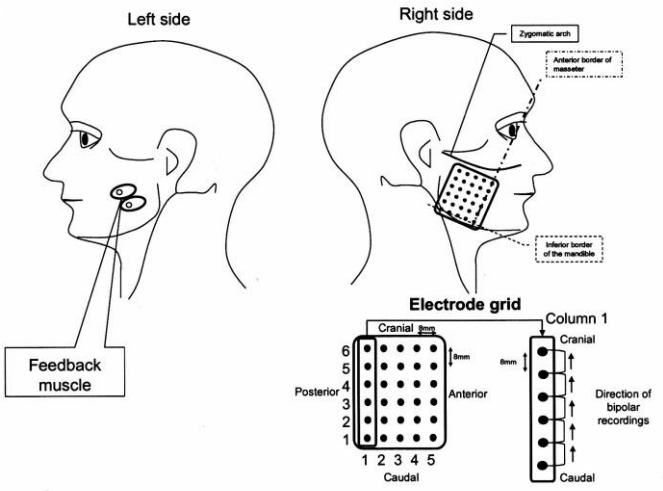

Figure 1. Schematic representation of the placement of the 5 × 6 electrode grid. For each column of electrodes, multi-channel bipolar recordings were obtained from adjacent electrodes in the caudal-cranial direction, providing 25 multi-channel bipolar recordings.

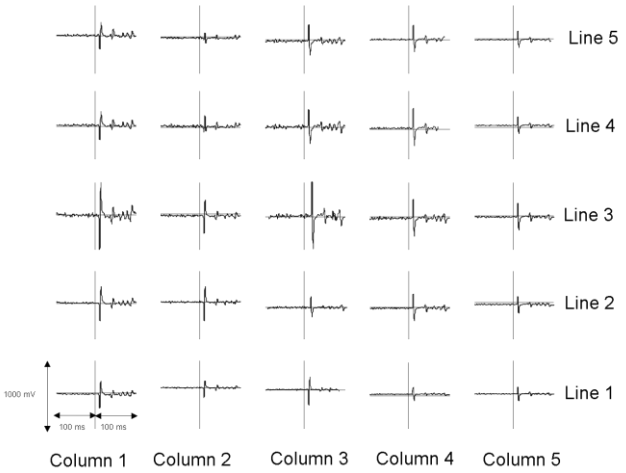

Figure 2. Example of the reflex response as recorded at the 25 recording sites over the right masseter of one subject. Each signal is the average of 20 sweeps.

Procedures

During the recording of the reflexes the subjects were seated upright in front of the muscle stretcher while they were biting with the incisors on the two jaw bars. The jaw bars were covered with a thin piece of rubber to protect the teeth and with a disposable small plastic coverage as a hygiene measure. The thickness of these two plastic layers was negligible and did not increase the initial vertical bite.

Each participant performed three, 5-s maximal clenches with the incisor teeth on the jaw bars of the muscle-stretcher. The mean value of the root mean square of the three maximum EMG recordings of the left masseter muscle was regarded as the maximum voluntary contraction (MVC) and was used for visual feedback for the remainder of the experiment. The participants were then instructed to contract their muscles at a level corresponding to 15% of the MVC. The muscle stretcher was automatically triggered each time the EMG activity of the left masseter was stable between 13.5% and 16.5% of the MVC value for 400 ms. For each stimulus and for each of the multichannel bipolar recordings, 300 ms of peristimulus EMG activity was recorded, with 100 ms prestimulus and 200 ms poststimulus. In total, 20 reflexes were evoked per participant.

Signal analysis

For each of the multichannel bipolar recordings, the EMG signal in the 100 ms preceding the stimulus was first full-wave rectified and then averaged. The mean of the 20 reflex sweeps was considered the prestimulus EMG activity of that bipolar recording.

For analysis of the reflex amplitude of each multi-channel bipolar recording, the EMG signals of the 200 ms following a stimulus were averaged over the 20 individual sweeps. The jaw-stretch reflex appeared as a biphasic potential in this averaged sweep (Scutter and Türker, 2001). The amplitude of this reflex was the peak-to-peak value of this biphasic potential. The reflex amplitude was also normalized (%) with respect to the mean pre-stimulus EMG activity of that multi-channel bipolar recording.

Statistical analysis

The mean pre-stimulus EMG activity, the mean reflex amplitude, and the mean normalized reflex amplitude were analyzed separately with two-way analysis of variance (ANOVA) with as factors the posterior-anterior and caudal-cranial direction. When sphericity was violated (i.e., a non-equal level of dependence between the axes was present), conservative Greenhouse-Geisser corrected values of the degree of freedom were used (Mauchly, 1940; Greenhouse, 1959). For each participant, a cross-correlation analysis was also performed between the 25 mean pre-stimulus EMG activities and the corresponding reflex amplitudes. Significance was accepted for $P < 0.05$ and results are presented as mean and standard deviation (mean ± SD).

RESULTS

The jaw-stretch reflex amplitude depended on the recording site (Fig. 2, Fig. 3A, and Table IA). The two-way ANOVA showed that this site dependency was statistically significant, both for the posterior-anterior ($P = 0.006$, $F = 5.6$) and caudal-cranial direction ($P = 0.001$, $F = 11.2$). An interaction was also found between the two directions ($P < 0.001$, $F = 5.2$). Also, the mean pre-stimulus EMG activity depended on the recording site, both in the posterior-anterior ($P < 0.001$, $F = 15.7$) and in the caudal-cranial directions ($P < 0.001$, $F = 9.1$) (Fig. 3B, Table IB). Again, an interaction was found between the two directions ($P = 0.005$, $F = 5.1$).

High cross-correlation coefficients were found between the 25 mean prestimulus EMG activities and reflex amplitudes (mean correlation coefficient over the 15 volunteers, 0.88 ± 0.17), indicating that the reflex and voluntary EMG activities had a similar spatial distribution over the muscle.

When the reflex amplitude was normalized with respect to the prestimulus EMG activity, no dependency was found for the posterior-anterior direction ($P = 0.26$, $F = 1.4$) or the caudal-cranial direction ($P = 0.68$, $F = 0.4$) (Fig. 3C and Table IC, II). Some site dependency was still present as indicated by the interaction between the two factors of the ANOVA which just reached statistical significance ($P = 0.05$, $F = 2.3$).

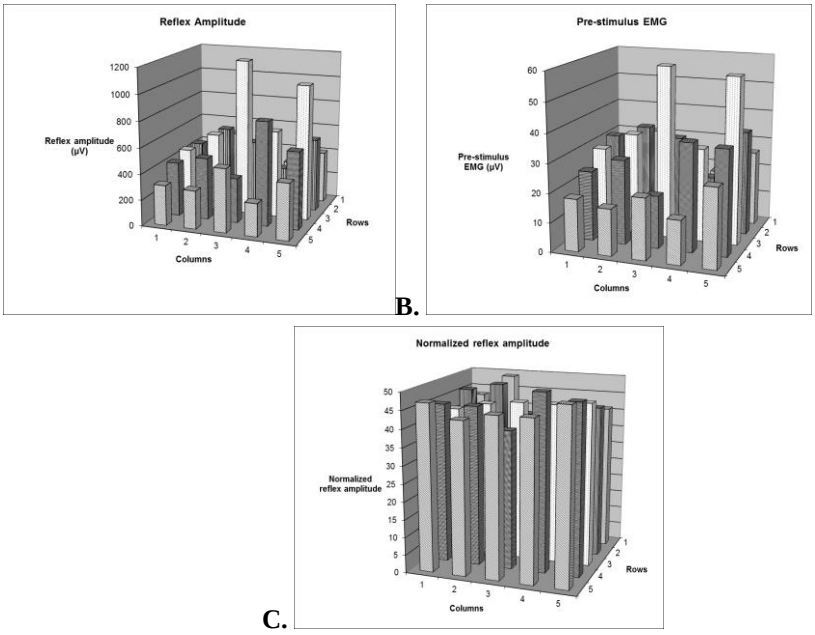

Figure 3. Schematic representation of the mean values (± SD, *n* = 15 subjects) of the reflex amplitude (μV) (**A**), the full-wave rectified and averaged (over 100 ms) pre-stimulus EMG activity (μV) (**B**), and the normalized reflex amplitude (**C**) (data presented in Table I).

Table 1. Mean values (± SD, *n* = 15 subjects) of the reflex amplitude (μV) **(A)**, the full-wave rectified and averaged (over 100 ms) pre-stimulus EMG activity (μV) **(B)**, and the normalized reflex amplitude **(C)**.

A.	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	311 ± 265	296 ± 304	491 ± 569	254 ± 193	433 ± 292
Row 4	418 ± 375	477 ± 439	344 ± 263	802 ± 893	598 ± 419
Row 3	459 ± 418	602 ± 466	1193 ± 1284	669 ± 663	1039 ± 778
Row 2	452 ± 387	591 ± 502	504 ± 306	313 ± 205	566 ± 369
Row 1	255 ± 187	500 ± 419	468 ± 336	299 ± 249	401 ± 257

B.	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	18 ± 16	16 ± 14	21 ± 21	15 ± 11	27 ± 19
Row 4	24 ± 24	29 ± 29	18 ± 12	37 ± 36	36 ± 26
Row 3	29 ± 29	35 ± 27	59 ± 56	32 ± 27	57 ± 43
Row 2	31 ± 36	35 ± 31	32 ± 21	19 ± 13	36 ± 24
Row 1	19 ± 13	31 ± 27	28 ± 20	18 ± 14	26 ± 19

C.	Column 1	Column 2	Column 3	Column 4	Column 5
Row 5	47 ± 12	43 ± 13	45 ± 16	45 ± 9	49 ± 7
Row 4	45 ± 14	45 ± 10	39 ± 10	50 ± 11	48 ± 11
Row 3	42 ± 13	44 ± 14	45 ± 14	45 ± 11	46 ± 11
Row 2	46 ± 12	48 ± 9	40 ± 12	38 ± 9	43 ± 12
Row 1	43 ± 14	49 ± 8	42 ± 15	39 ± 14	41 ± 12

Table 2. Results from the two-way ANOVA on the data presented in Table I. (* statistically significant results).

	Caudal-cranial direction		Posterior-Anterior direction		Interaction	
	F	p-value	F	p-value	F	p-value
Reflex amplitude	11.2	0.001*	5.6	0.006*	5.2	< 0.001*
Pre-stimulus EMG activity	9.1	< 0.001*	15.7	< 0.001*	5.1	0.005*
Normalized reflex amplitude	0.4	0.68	1.4	0.26	2.3	0.05

DISCUSSION

The results of this study showed that, when the jaw-stretch reflex amplitude of the masseter muscle is normalized with respect to the pre-stimulus EMG amplitude, it becomes independent of the location of the electrodes over the muscle.

Human jaw-stretch reflexes are extensively studied because they give insight into the neurophysiologic trigeminal mechanisms responsible for the motor control of jaw-closing muscles (Poliakov and Miles, 1994). The monosynaptic jaw-stretch reflex is part of the response to a sudden perturbation that activates the muscle stretch-sensitive receptors. Two methods have been proposed for quantifying the excitatory part of the reflex. The first method uses the peak-to-peak amplitude of the biphasic potential appearing in the EMG signal (Biasiotta et al., 2007; Kimura et al., 1994; Lobbezoo et al., 2003; Murray and Klineberg, 1984; Peddireddy et al., 2005; 2006; Svensson et al., 2000; Svensson et al., 2001; Svensson et al., 2003; Türker, 2002; van Selms et al., 2005; Wang et al., 2000; Wang and Svensson, 2001), whereas the second one uses only the first depolarizing peak (Widmer et al., 1989;Lobbezoo et al., 1993a; b; Lobbezoo et al., 1993c; 1996). In the present study, the first method was used for analysis of the data. Separate analysis using the second method led to the same results (data not shown).

The development of highly controlled jaw-muscle stretchers (Miles et al., 1993; Svensson et al., 2000) has enabled the provocation of the jaw-stretch reflex under standardized conditions. With the use of such equipment, it has been shown that the reflex amplitude is proportionally dependent on the level of the pre-stimulus EMG amplitude (Wang and Svensson, 2001), making it necessary to control the excitability of the motoneuronal pool during reflex provocation (Türker, 2007). In the present experiment, in order to take this into account, the level of background EMG amplitude was controlled through visual feedback from the left masseter muscle.

The EMG activity in the masseter muscle has been reported to vary between different recording sites (Schumann et al., 1994; Castroflorio et al., 2005). The results of the present study show that a similar degree of variability exists in the amplitude of the jaw-stretch reflex. This may substantially increase the variability of results among studies which use different criteria for electrode placement. The high cross-correlation coefficients between the pre-stimulus EMG amplitude and the reflex amplitude imply that muscle areas with high voluntary EMG amplitude also show high amplitudes of the stretch-reflex. Anatomical and physiological factors, such as the dispersed innervation zones of the masseter muscle (Castroflorio et al., 2005) and the relatively small motor unit territories in relation to limb muscles (van Eijden and Turkawski, 2001), may account for these similar spatial distributions. Normalization of the reflex by the pre-stimulus EMG activity reduced its spatial dependency and made the recording of the reflex independent of the location of the electrodes. Thus, it would be expected that the reproducibility of the recording of the normalized reflex amplitude would be increased.

In conclusion, in young, healthy participants, normalization of the jaw-stretch reflex amplitude by the pre-stimulus EMG amplitude strongly reduces the spatial variability typical of non-normalized reflex responses.

REFERENCES

Biasiotta A, Peddireddy A, Wang K, Romaniello A, Frati A, Svensson P *et al.* (2007). Effect of pinching-evoked pain on jaw-stretch reflexes and exteroceptive suppression periods in healthy subjects. *Clin Neurophysiol* 118(10)*:*2180-2188.

Castroflorio T, Farina D, Bottin A, Piancino MG, Bracco P, Merletti R (2005). Surface EMG of jaw elevator muscles: effect of electrode location and inter-electrode distance. *J Oral Rehabil* 32(6)*:*411-417.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6(4)*:*301-355.

Greenhouse SW, Geisser S (1959). On methods in the analysis of profile data. *Psychometrika* 24(95-111.

Kimura J, Daube J, Burke D, Hallett M, Cruccu G, Ongerboer de Visser BW *et al.* (1994). Human reflexes and late responses. Report of an IFCN committee. *Electroencephalography and clinical neurophysiology* 90(6)*:*393-403.

Lobbezoo F, van der Glas HW, Buchner R, van der Bilt A, Bosman F (1993a). Gain and threshold of the jaw-jerk reflex in man during isometric contraction. *Exp Brain Res* 93(1)*:*129-138.

Lobbezoo F, van der Glas HW, Buchner R, van der Bilt A, Bosman F (1993b). Jaw-jerk reflex activity in relation to various clenching tasks in man. *Exp Brain Res* 93(1)*:*139-147.

Lobbezoo F, van der Glas HW, van der Bilt A, Buchner R, Bosman F (1993c). Bilateral asymmetries in the jaw-jerk reflex activity in man. *Arch Oral Biol* 38(8)*:*689-698.

Lobbezoo F, van der Glas HW, van der Bilt A, Buchner R, Bosman F (1996). Sensitivity of the jaw-jerk reflex in patients with myogenous temporomandibular disorder. *Arch Oral Biol* 41(6)*:*553-563.

Lobbezoo F, Wang K, Aartman IH, Svensson P (2003). Effects of TMJ anesthesia and jaw gape on jaw-stretch reflexes in humans. *Clin Neurophysiol* 114(9)*:*1656-1661.

Lund JP, Lamarre Y, Lavigne G, Duquet G (1983). Human jaw reflexes. *Advances in neurology* 39(739-755.

Mauchly JW (1940). Significance test for sphericity of a normal *n*-variate distribution. *Ann Math Stat* 11(204-209.

Miles TS, Poliakov AV, Flavel SC (1993). An apparatus for controlled stretch of human jaw-closing muscles. *J Neurosci Methods* 46(3)*:*197-202.

Miles TS (2007). Postural control of the human mandible. *Arch Oral Biol* 52(4)*:*347-352.

Murray GM, Klineberg IJ (1984). A standardized system for evoking reflexes in human jaw elevator muscles. *J Oral Rehabil* 11(4)*:*361-372.

Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L (2005). Effect of experimental posterior temporalis muscle pain on human brainstem reflexes. *Clin Neurophysiol* 116(7)*:*1611-1620.

Peddireddy A, Wang K, Svensson P, Arendt-Nielsen L (2006). Influence of age and gender on the jaw-stretch and blink reflexes. *Exp Brain Res* 171(4):530-540.

Poliakov AV, Miles TS (1994). Stretch Reflexes in Human Masseter. *J Physiol-London* 476(2):323-331.

Schumann NP, Scholle HC, Anders C, Mey E (1994). A topographical analysis of spectral electromyographic data of the human masseter muscle under different functional conditions in healthy subjects. *Arch Oral Biol* 39(5):369-377.

Scutter SD, Turker KS (2001). The role of the muscle spindles in human masseter. *Human movement science* 20(4-5):489-497.

Svensson P, Miles TS, Graven-Nielsen T, Arendt-Nielsen L (2000). Modulation of stretch-evoked reflexes in single motor units in human masseter muscle by experimental pain. *Exp Brain Res* 132(1):65-71.

Svensson P, Macaluso GM, De Laat A, Wang K (2001). Effects of local and remote muscle pain on human jaw reflexes evoked by fast stretches at different clenching levels. *Exp Brain Res* 139(4):495-502.

Svensson P, Wang K, Arendt-Nielsen L (2003). Effect of muscle relaxants on experimental jaw-muscle pain and jaw-stretch reflexes: a double-blind and placebo-controlled trial. *Eur J Pain* 7(5):449-456.

Türker K (2007). Exteroceptive suppression of the jaw closing muscle electromyogram: methodological issues applicable to all human reflex studies. *Clin Neurophysiol* 118(951-953.

Turker KS (2002). Reflex control of human jaw muscles. *Crit Rev Oral Biol M* 13(1):85-104.

van Eijden TM, Turkawski SJ (2001). Morphology and physiology of masticatory muscle motor units. *Crit Rev Oral Biol Med* 12(1):76-91.

van Selms MK, Wang K, Lobbezoo F, Svensson P, Arendt-Nielsen L, Naeije M (2005). Effects of masticatory muscle fatigue without and with experimental pain on jaw-stretch reflexes in healthy men and women. *Clin Neurophysiol* 116:1415-1423.

Wang K, Svensson P, Arendt-Nielsen L (2000). Effect of tonic muscle pain on short-latency jaw-stretch reflexes in humans. *Pain* 88(2):189-197.

Wang K, Svensson P (2001). Influence of methodological parameters on human jaw-stretch reflexes. *Eur J Oral Sci* 109(2):86-94.

Widmer CG, Lund JP (1989). Evidence that peaks in EMG averages can sometimes be caused by inhibition of motorneurons. *J Neurophysiol* 62(1):212-219.

Chapter 4

Provocation of delayed-onset muscle soreness in the human jaw-closing muscles

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Arch Oral Biol 2010; 55(9):621-626

ABSTRACT

Eccentric contractions of jaw-closing muscles are difficult to perform. This may explain why fatigue-inducing experiments performed so far suggest the jaw-closing muscles to be fatigue-resistant. Aim of this study was to construct an apparatus that can impose intense eccentric contractions to the jaw-closing muscles, and to test the hypothesis that eccentric contractions can provoke symptoms of delayed-onset muscle soreness (DOMS) in these muscles.

The provocation apparatus consists of two tungsten arms connected by a hinge axis on one end. Participants bite with their anterior teeth on biting plates located on the other end. Each time the experimenter gradually releases the compression force of the apparatus' rubber tubings, the mouth is forced open and the jaw-closing muscles perform an eccentric contraction. Six male participants performed eccentric contractions of their jaw-closing muscles in six sets of exercises, each lasting 5 minutes, and with one minute of rest in between. Each set consisted of 60 open-close movements. Before and after the exercises, and after 24 hours, 48 hours, and one week, feelings of fatigue and pain, the maximum mouth opening without pain, muscles' tenderness to palpation and the maximum voluntary bite force were recorded.

After 24 hours and 48 hours, the levels of fatigue and pain were elevated, the maximum mouth opening without pain was smaller, and five of the participants reported tenderness to palpation. The maximum voluntary bite force was also smaller after 24 hours. These findings indicate that this novel apparatus is successful in inducing DOMS in the jaw-closing muscles.

Key words: eccentric exercises, delayed-onset muscle soreness (DOMS), human jaw-closing muscles, jaw-muscle fatigue, jaw-muscle pain, visual analogue scale (VAS), maximum mouth opening, maximum voluntary bite force

ACKNOWLEDGEMENTS

This study is supported by the European Union Marie Curie Chair Project (GenderReflex; MEX-CT-2006-040317), Turkish Scientific and Technical Research Organization (TÜBİTAK; 107S029 - SBAG-3556), and the Netherlands Institute for Dental Sciences (IOT).

INTRODUCTION

Human jaw muscles are of vital importance for the biological and social well-being of humans. They act as a group of jaw closers or jaw openers and are important for functions like chewing, talking, drinking, and smiling, which are essential for the human race (Creanor and Noble, 1994).

Jaw-closing muscles are considered to differ in several aspects from muscles in other parts of the human body. Human jaw-closing muscles contain large, homogeneous groups of fibers. The diameter of the type I fibers are greater than those of type II fibers, but both are comparatively small. This contrasts strongly with the limb and trunk muscles, where there are mosaic-like, mixed fiber distributions, and where fiber diameter is correlated to histochemical type (Hannam and McMillan, 1994; van Eijden and Turkawski, 2001). Moreover, adult masseter muscles also contain neonatal myosin heavy-chain isozymes and alpha-cardiac myosin, which have not been described in normal limb muscles (Hannam and McMillan, 1994).

Intense dynamic (Farella et al., 2001) and isometric (Svensson et al., 2001) contractions have been successful in inducing short-lasting fatigue and pain in the jaw-closing muscles, which rapidly disappear after cessation of the exercise. A variety of protocols, like mimicking tooth-grinding activity (Arima et al., 1999) and resisted opening and closing mouth movements (Svensson and Arendt-Nielsen, 1995), have been used to provoke delayed-onset muscle soreness (DOMS) in the jaw-closing muscles. However, these protocols were only successful in provoking mild symptoms of DOMS in these muscles. From limb muscles, it is known that DOMS is especially provoked by intense eccentric contractions of the muscles involved (Cleak and Eston, 1992; Kroon and Naeije, 1991). During eccentric exercise, the contracting muscle is forcibly lengthened (Proske and Morgan, 2001). Due to the anatomy of the masticatory system, eccentric contractions of the jaw-closing muscles are difficult to perform. This may explain why the results of fatigue and pain inducing experiments performed so far suggest the jaw-closing muscles to be fatigue resistant (Arima et al., 1999; Svensson et al., 2001).

Therefore, the aim of the present study was to construct an apparatus capable of imposing intense eccentric contractions to the jaw-closing muscles, and to test the hypothesis that repetitive eccentric contractions can provoke symptoms of DOMS in the jaw-closing muscles.

MATERIALS AND METHODS

Provocation apparatus

The provocation apparatus (Figure 1) consists of two tungsten arms connected by a hinge axis on one end and with two biting plates on the other end. In each arm six grooves and five holes were drilled. Opposing grooves offer the possibility to place pieces of rubber tubing between the two arms. A lever, connected to a bolt which also connects the two arms

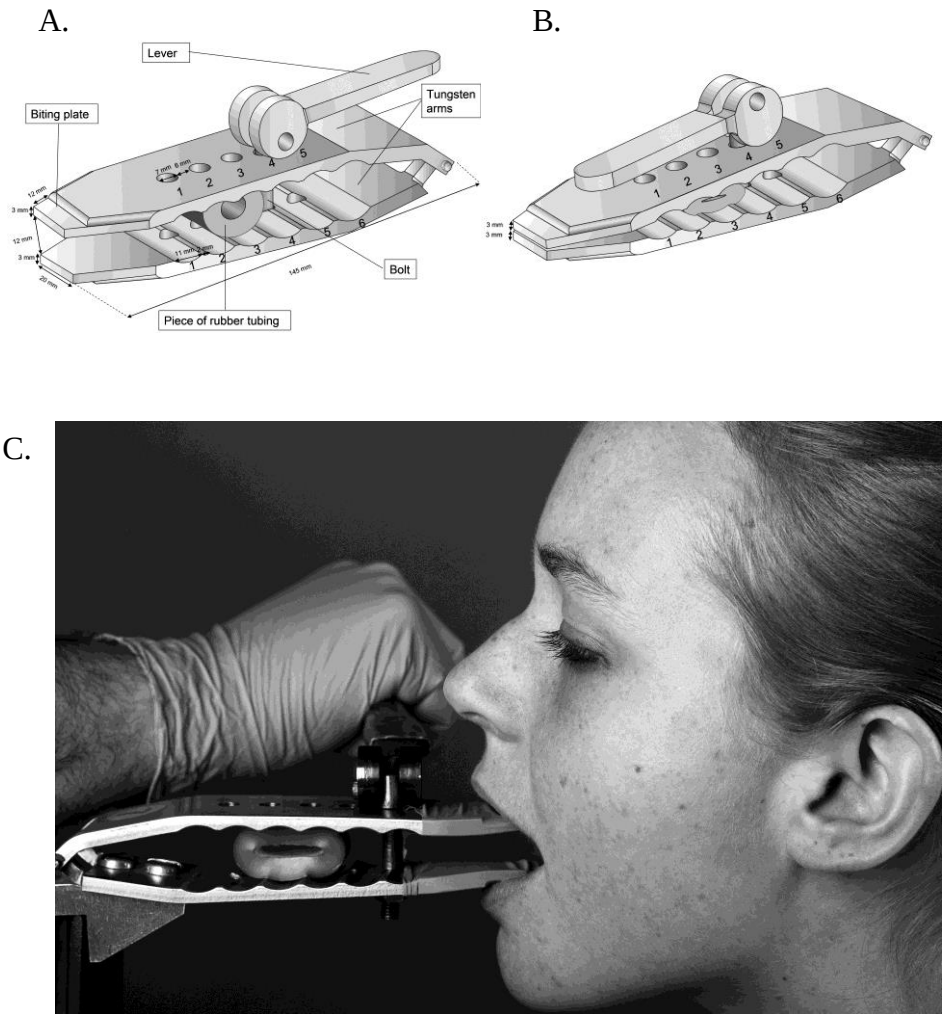

Figure 1. Eccentric Contraction Apparatus: A. Open and B. Closed. C. Participants placed their teeth on the upper and lower biting plates of the apparatus in the 'closed' position, and were instructed to continuously bite at a predetermined biting level. When the experimenter released the compressed tension of the rubber tubings, the contraction apparatus opened and the jaw-closing muscles were eccentrically contracting.

by its placement through two opposing holes, enables to vary the distance between the two biting plates. When the device is closed by rotating the lever by 180 degrees, an elastic force is built up in the rubber tubing. When the lever is gradually rotated backwards to its original position, this elastic force will urge the arms of the apparatus to open and to overcome the biting force exerted by the participant on the two biting plates. The jaw-closing muscles of the participant will therefore perform an eccentric contraction.

Dependent upon the biting force exerted by the participant, one or two pieces of rubber tubing are placed in opposing grooves closer or farther away from the hinge axis of the device. The thickness of each metal biting plate is 3 mm and, at maximum seperation, the two arms are 12 mm apart, thus yielding an interincisal distance of 18 mm.

Experimental protocol

The provocation apparatus was tested in an experimental protocol aimed at provoking DOMS in the human jaw-closing muscles. Sets of data were collected at baseline, immediately after the provocation, and 24 hours, 48 hours, and one week later on.

Each data set consisted of measurements of fatigue and pain in the jaw-closing muscles, of the maximum mouth opening without pain, of the tenderness to palpation of the jaw-closing muscles, and of the maximum voluntary bite force (MVBF).

Subjective feelings of fatigue and pain in the jaw-closing muscles were rated at two separate 100 mm visual analogue scales (VAS), with left anchor words "No fatigue/pain at all" and right anchor words "Fatigue/Pain as worse as it could be". Participants were asked to draw a perpendicular line at the point representing their subjective feelings of fatigue and pain, respectively.

Maximum mouth opening was recorded by asking participants to open their mouths as wide as possible, without experiencing any pain. The distance between the incisal edges of the right upper central incisor and the right lower central incisor were added to the vertical overjet to come to the maximum mouth opening without pain (MMO without pain).

The major jaw-closing muscles, the masseter and temporalis, were manually palpated (Dworkin and LeResche, 1992). The temporalis was palpated on three points (its posterior, middle, and anterior part) and the masseter was palpated on its origin, body, and insertion.

MVBF was recorded by asking the participants to bite as hard as possible on a custom-made bite force transducer. Four efforts, lasting 10s each, were performed and the MVBF was the maximum value of these four efforts. During each effort, participants were verbally encouraged to increase their biting force gradually to its maximum, then to keep it at that level for approximately 3 seconds and then to relax. In order to protect the teeth from possible damage, individual impressions of both upper and lower teeth were made with a dental putty material (PROVIL® novo; Heraeus Kulzer, Hanau, Germany). In order to avoid muscle fatigue, the clenching efforts were separated by 2 minutes of rest.

Provocation part

During the provocation part, eccentric contractions of the jaw-closing muscles were performed in six sets of exercises, each lasting 5 minutes, and with one minute of rest in between. Each set of exercises consisted of about 60 open-close movements, each movement lasting about 5 seconds.

The provocation apparatus was mounted on a table with the fixed lower arm parallel to the floor of the experimental room. Participants were sitting upright in a chair with an adjustable height, so that they could comfortably bite with their central incisors on the biting plates of the apparatus. In order to protect the teeth from pain or possible fractures, participants were wearing soft acrylic mouth guards (Bioplast, Ref 3188.1, 4,0x125mm, clear, Scheu Dental Technology, Am Burgberg 20, 58642 Iserlohn, Germany), made on individual plaster casts of both dental arches. Two pieces of rubber tubing were used between the two metal arms throughout the provocation. The release of the lever was done at a steady pace controlled by the experimenter, under the guidance of a silent metronome.

During the open-close movements, participants were constantly biting at a level of 30% of the baseline maximum electromyographic (EMG) activity of their right masseter muscle. The root mean square values of the EMG activity were displayed to the participant through visual feedback with the use of a voltmeter. Custom-made, bipolar surface electrodes made of silver amalgam (diameter: 4 mm) were placed over the belly of the right masseter muscle after thorough cleaning of the skin with alcohol. The electrodes were placed parallel to the muscle fibres and with their centres approximately 15 mm apart. The EMG signals were filtered (bandwidth 5-1000 Hz), amplified (1000 times), and sampled (2000 Hz). An isolated metal common electrode held in the palm of the hand served as a reference electrode.

Participants

Six healthy male participants (mean age ± SD = 35,67 ± 13,49 years) with no history of temporomandibular disorders (TMD) or chronic orofacial pain complaints participated in the study. Before the start of the experiment, they gave a written informed consent. The study was conducted according to the Helsinki declaration and the protocol was approved by the University of Adelaide Human Research Ethics Committee.

Statistical analysis

The mean VAS scores, the mean MMO without pain, and the mean MVBF values were analysed with repeated measures analysis of variance (ANOVA) with time as a within-participants factor with five levels (before, after, after 24 hours, after 48 hours, after one week). Pair-wise comparisons were performed with simple contrast analysis and the first (before) level as reference.

RESULTS

Immediately after the provocation part, participants reported significantly elevated levels of fatigue and pain ($p<0.01$ for both) that remained elevated at the follow-up appointments after 24 and 48 hours ($p<0.05$). After one week, fatigue and pain VAS values did not differ anymore from baseline (Figure 2).

The MMO without pain was significantly decreased at the appointments 24 hours ($p<0.01$) and 48 hours ($p<0.05$) after the provocation part when compared with baseline (Figure 3), indicating a restricted range of movement of the mandible.

Regarding the response to manual palpation of the jaw-closing muscles, three participants developed painful reactions on at least one palpated point immediately after the provocation part, while five participants showed painful reactions at the follow-up measurements 24 hours and 48 hours later on. At baseline and one week after the exercises, no painful palpation points were found (Figure 4).

Compared to baseline, the MVBF was significantly decreased at the follow-up appointment 24 hours after the provocation part ($p<0.05$) (Figure 5).

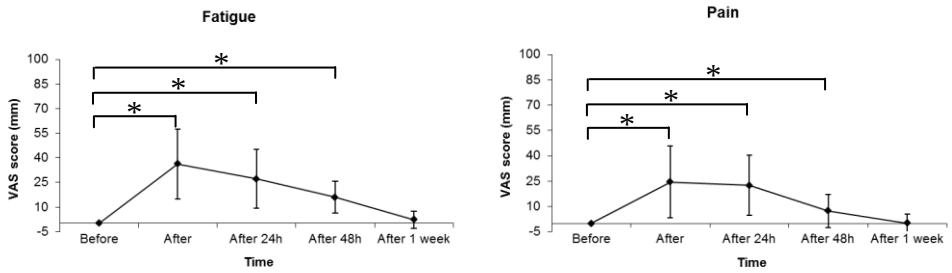

Figure 2. Mean values ± SD regarding the subjective feelings of fatigue and pain, rated at two separate 100-mm VAS, at baseline, immediately after the provocation, and 24 hours, 48 hours, and one week later on (*: $p<0.05$; **: $p<0.01$).

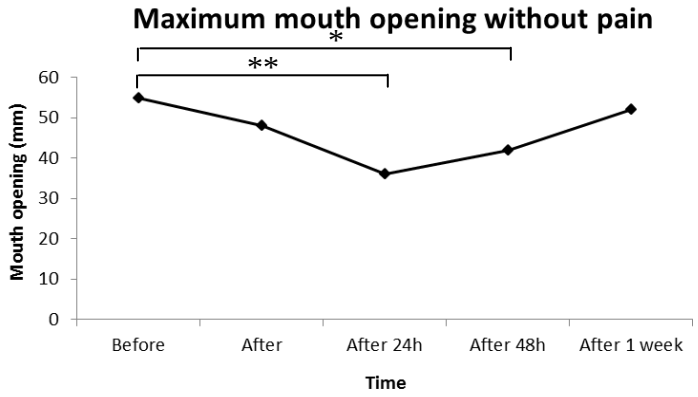

Figure 3. Mean values ± SD of the maximum mouth opening without pain, recorded at baseline, immediately after the provocation, and 24 hours, 48 hours, and one week later on (*: $p<0.05$; **: $p<0.01$).

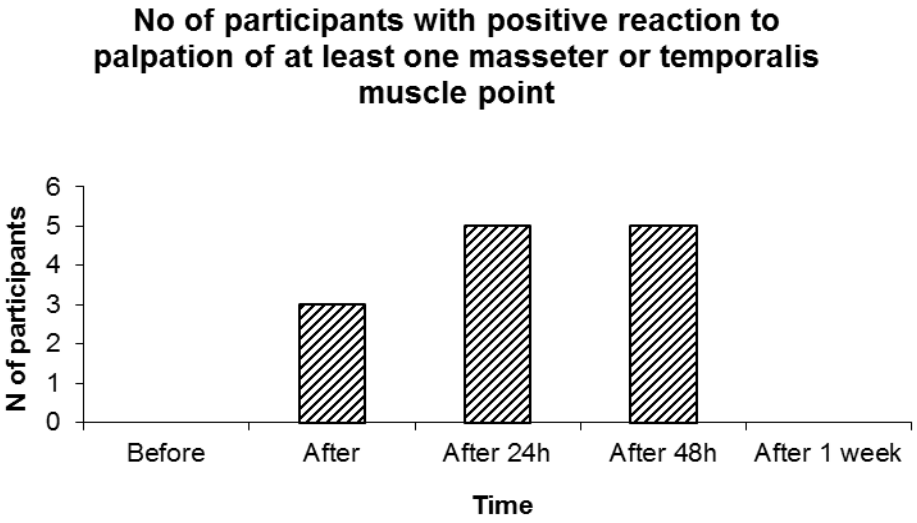

Figure 4. Number of participants with at least one painful reaction to manual palpation of the masseter and temporalis muscles, recorded at baseline, immediately after the provocation, and 24 hours, 48 hours, and one week later on.

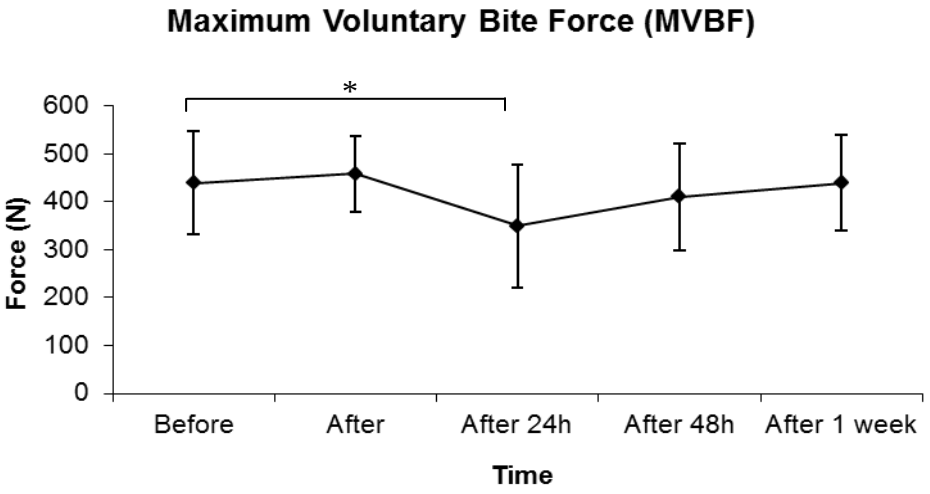

Figure 5. Mean values ± SD of the maximum voluntary bite force (MVBF), recorded at baseline, immediately after the provocation, and 24 hours, 48 hours, and one week later on (*: $p<0.05$).

DISCUSSION

In this study, an apparatus was presented which is capable of imposing intense eccentric contractions upon the jaw-closing muscles, and the hypothesis was tested that repetitive eccentric contractions imposed by this apparatus, can provoke symptoms of DOMS in the jaw-closing muscles.

Eccentric contractions can generate DOMS in limb muscles (Cheung et al., 2003; Proske and Morgan, 2001). It is characteristic for DOMS that it is experienced in the first 12-48 hours following intense eccentric exercise. DOMS presents with a variety of symptoms that include increased perceived muscle pain or soreness, reports of pain upon muscle palpation, a reduced maximum voluntary muscle force, and a limited range of motion (Prasartwuth et al., 2005). Although there is no consensus regarding the underlying mechanisms of DOMS (Clarkson and Sayers, 1999), inflammatory processes (Jones et al., 1986; Round et al., 1987), muscle fibre degeneration (Friden et al., 1983; Newham et al., 1983), and necrosis (Friden and Lieber, 1998; Nosaka and Clarkson, 1996) are typically suggested.

In the present study, signs and symptoms, typical for DOMS, could also be provoked in the jaw-closing muscles. This suggests that the conclusion drawn from pain and fatigue inducing experiments performed so far, viz., that the jaw-closing muscles are rather fatigue resistant (Arima et al., 1999; Svensson et al., 2001), may be due to difficulties imposing eccentric contractions to these muscles. The apparatus presented in this study has overcome this problem since it induces only eccentric contractions of the jaw-closing muscles and not combination of concentric and eccentric ones like a previous protocol (Svensson and Arendt-Nielsen, 1995). Its use in a DOMS provocation protocol has indicated that even though jaw-closing muscles differ from other skeletal muscles, they respond similarly to intense eccentric exercise.

The characteristics of DOMS are similar to the signs and symptoms of patients suffering from a myofascial temporomandibular disorder (TMD) pain. The aetiology of myofascial TMD pain is still unclear (Suvinen et al., 2005). Although bruxing habits, such as frequent clenching and grinding, are thought to play an important role in this respect, not every myofascial TMD patient is a frequent bruxer, indicating that other factors are involved as well (Lobbezoo and Lavigne, 1997). That myofascial TMD-like symptoms can be provoked in an experimental setting offers the possibility to gain more insight into the onset and aetiology of myofascial TMD pain.

In conclusion, the apparatus described in the present paper can successfully provoke DOMS in the jaw-closing muscles of healthy volunteers. This opens the possibility to further study the onset and aetiology of myofascial TMD pain.

REFERENCES

Arima T, Svensson P, Arendt-Nielsen L (1999). Experimental grinding in healthy subjects: a model for postexercise jaw muscle soreness? *J Orofac Pain* 13(2)*:*104-114.

Cheung K, Hume P, Maxwell L (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 33(2)*:*145-164.

Clarkson PM, Sayers SP (1999). Etiology of exercise-induced muscle damage. *Can J Appl Physiol* 24(3)*:*234-248.

Cleak MJ, Eston RG (1992). Delayed onset muscle soreness: mechanisms and management. *J Sports Sci* 10(4)*:*325-341.

Creanor S, Noble H (1994). Comparative functional anatomy. In: Temporomandibular joint and masticatory muscle disorders. GEC George A. Zarb, Barry J. Sessle, Norman D. Mohl editor. Copenhagen: Munksgaard.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6(4)*:*301-355.

Farella M, Bakke M, Michelotti A, Martina R (2001). Effects of prolonged gum chewing on pain and fatigue in human jaw muscles. *Eur J Oral Sci* 109(2)*:*81-85.

Friden J, Sjostrom M, Ekblom B (1983). Myofibrillar damage following intense eccentric exercise in man. *Int J Sports Med* 4(3)*:*170-176.

Friden J, Lieber RL (1998). Segmental muscle fiber lesions after repetitive eccentric contractions. *Cell Tissue Res* 293(1)*:*165-171.

Hannam AG, McMillan AS (1994). Internal organization in the human jaw muscles. *Crit Rev Oral Biol Med* 5(1)*:*55-89.

Jones DA, Newham DJ, Round JM, Tolfree SE (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *J Physiol* 375(435-448.

Kroon GW, Naeije M (1991). Recovery of the human biceps electromyogram after heavy eccentric, concentric or isometric exercise. *Eur J Appl Physiol Occup Physiol* 63(6)*:*444-448.

Lobbezoo F, Lavigne GJ (1997). Do bruxism and temporomandibular disorders have a cause-and-effect relationship? *J Orofac Pain* 11(1)*:*15-23.

Newham DJ, McPhail G, Mills KR, Edwards RH (1983). Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci* 61(1)*:*109-122.

Nosaka K, Clarkson PM (1996). Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med Sci Sports Exerc* 28(8)*:*953-961.

Prasartwuth O, Taylor JL, Gandevia SC (2005). Maximal force, voluntary activation and muscle soreness after eccentric damage to human elbow flexor muscles. *J Physiol* 567(Pt 1)*:*337-348.

Proske U, Morgan DL (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 537(Pt 2)*:*333-345.

Round JM, Jones DA, Cambridge G (1987). Cellular infiltrates in human skeletal muscle: exercise induced damage as a model for inflammatory muscle disease? *J Neurol Sci* 82(1-3)*:*1-11.

Suvinen TI, Reade PC, Kemppainen P, Kononen M, Dworkin SF (2005). Review of aetiological concepts of temporomandibular pain disorders: towards a biopsychosocial model for integration of physical disorder factors with psychological and psychosocial illness impact factors. *Eur J Pain* 9(6)*:*613-633.

Svensson P, Arendt-Nielsen L (1995). Effect of topical NSAID on post-exercise jaw muscle soreness: a placebo-controlled experimental study. *J Musculoskel Pain* 3(4)*:*41-58.

Svensson P, Burgaard A, Schlosser S (2001). Fatigue and pain in human jaw muscles during a sustained, low-intensity clenching task. *Arch Oral Biol* 46(8)*:*773-777.

van Eijden TM, Turkawski SJ (2001). Morphology and physiology of masticatory muscle motor units. *Crit Rev Oral Biol Med* 12(1)*:*76-91.

Chapter 5

Is myofascial pain in the temporomandibular disorder patients a manifestation of delayed-onset muscle soreness?

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Clin J Pain: 2013;29(8):712-716

ABSTRACT

In a study to the possible role of overuse of jaw muscles in the pathogenesis of jaw-muscle pain, a protocol involving concentric and eccentric muscle contractions was used to provoke a state of delayed onset of muscle soreness (DOMS) in the jaw muscles of healthy individuals. It was tested whether the accompanying signs and symptoms would yield the temporary diagnosis of myofascial pain according to the research diagnostic criteria for temporomandibular disorders (RDC-TMD) in these individuals. Forty persons (mean age ± SD = 27.7 ± 7.5 years) performed six, 5-min bouts of eccentric and concentric jaw-muscle contractions. Before and immediately after the exercise, and 24 hours, 48 hours, and one week later, self-reported muscle fatigue and pain, pain-free maximum mouth opening, pressure-pain thresholds, and the number of painful jaw-muscle palpation sites were recorded. Significant signs and symptoms of DOMS in the jaw muscles were found, which all had resolved after a week. In 31 (77.5%) of the participants, these signs and symptoms also gave rise to a temporary diagnosis of myofascial pain according to the RDC-TMD. Within the limits of the study it was shown that an experimental protocol involving concentric and eccentric muscle contractions can provoke a DOMS in the jaw muscles and the temporary diagnosis of myofascial pain according to the RDC-TMD. This strengthens the suggestion that the myofascial pain in TMD patients may be a manifestation of DOMS in the jaw muscles.

ACKNOWLEDGMENTS

This study is supported by the European Union Marie Curie Chair Project (GenderReflex;MEX-CT-2006-040317), Turkish Scientific and Technical Research Organization (TÜBİTAK;107S029-SBAG-3556), and the Netherlands Institute for Dental Sciences (IOT). The authors are sincerely grateful to Corine Visscher for her statistical advice.

INTRODUCTION

Chronic musculoskeletal pain, such as low back and neck pain, is a common symptom in the general population (Wijnhoven et al., 2006), affecting the quality of life of its sufferers. In the orofacial region, the umbrella term "temporomandibular disorders" (TMD) (McNeil, 1993) is often used to denote musculoskeletal disorders in the jaw muscles and/or the temporomandibular joints. TMD pain has a prevalence of about 10% in the general population and affects females the most (LeResche, 1997). Jaw-muscle pain is the most common type of TMD pain in TMD patients (Manfredini et al., 2011). It often has a chronic character and includes the characteristics of pain at rest, and pain which exacerbates during jaw functions such as chewing, biting, and yawning. Moreover, the painful jaw muscles show a tenderness to palpation. The orofacial pain with these signs and symptoms is given the diagnosis of myofascial pain according to the internationally accepted research diagnostic criteria for TMD (RDC-TMD) (Dworkin and LeResche, 1992).

The pathogenesis of jaw-muscle pain in TMD patients is not yet well understood. Epidemiological studies indicate that adverse bruxing habits such as frequent clenching and grinding may play an important role in initiating and perpetuating jaw-muscle pain (Svensson et al., 2008). However, most of the experimental muscle-exercise protocols performed so far have only been successful in provoking a transient jaw-muscle pain that was especially present during the exercise and that resolved quickly after its cessation (Svensson et al., 2008). This may question the role of overuse of the jaw muscles in the pathogenesis of jaw-muscle pain in TMD patients, or it may indicate that the protocols used were not successful in mimicking the overuse of the jaw muscles as present in TMD patients. However, experimental protocols especially involving eccentric jaw muscle contractions were successful in provoking a jaw-muscle pain that did not resolve quickly after the exercise but that lingered on until a few days after the exercise (Svensson and Arendt-Nielsen, 1995; Svensson et al., 1997; Türker et al., 2010; Torisu et al., 2008; Torisu et al., 2010). Muscle pain being present for a few days after the exercise is a well-known phenomenon in limb muscles and is usually called a delayed onset muscle soreness (DOMS) (Cheung et al., 2003). During the oral habit of teeth grinding, the lower jaw performs a high number of consecutive small lateral or protrusive movements, with teeth contact. The jaw muscles, which are tonically active during these grinding movements, then undergo concentric and eccentric contractions (Kato et al., 2003a) and this raises the question whether the jaw-muscle pain experienced by TMD patients is a manifestation of DOMS in the jaw muscles.

Therefore, to further study the possible role of overuse of jaw muscles in the pathogenesis of jaw-muscle pain, a protocol involving concentric and eccentric contractions of the jaw muscles was used to provoke a state of DOMS in the muscles of healthy individuals. It was hypothesized that the accompanying signs and symptoms would yield

the temporary diagnosis of myofascial pain according to the RDC-TMD in these individuals. If so, this would strengthen the suggestion that the jaw-muscle pain in TMD patients is a manifestation of DOMS in the jaw muscles.

METHODS

Participants

After receiving detailed information about the experiment, 40 volunteers (20 males, 20 females; mean age±SD=27.7±7.5 years) gave their written informed consent. All participants were healthy, did not use any medication, and had no dental pain pathology (de Leeuw, 2008). Individuals who reported oral parafunctions (i.e., tooth grinding, tooth clenching) were excluded. The experiments were conducted according to the Helsinki declaration, and the protocol was approved by the Clinical Research Ethics Committee of the Medical Faculty at Ege University.

Protocol Overview

The protocol consisted of baseline measurements, a provocation part, and data collection immediately, 24 hours, 48 hours, and 1 week after the provocation part. Data were collected regarding the subjective feelings of fatigue and pain intensity, the pain-free maximum mouth opening (MMO), the pressure-pain thresholds (PPTs) of the major jaw-closing muscles, and the number of painful muscle palpation sites.

Provocation part

During the provocation part, participants performed six sets of eccentric and concentric contractions, each set lasting 5 minutes, and with 1 minute of rest in between. During a set, the participants were continuously biting with their anterior teeth on one end of a custom-made jaw-muscle stretcher (Türker et al., 2010). The stretcher (Figure 1) consisted of two tungsten arms connected by a hinge axis on one end and two small biting plates on the other end. Opposing grooves enabled the placement of a rubber tubing between the two arms. When the compression force of the rubber tubing was gradually released by the experimenter, the participant's mouth was gently forced open, allowing jaw-closing muscles to contract eccentrically during approximately 3 sec. Subsequently, the experimenter manually closed the apparatus (the concentric contraction) (duration: 1 sec) and the compression force was built up again in the rubber tubing. The "occlusal" phase, i.e. the transition from closing to opening, took about 1 sec and thus, each open-close cycle lasted approximately 5 sec in total. This way, the participants performed about 60 eccentric and concentric contractions during each set. Participants continuously received visual feedback of the root mean square values of the electromyographic (EMG) activity of their right masseter muscle by means of a voltmeter. The EMG activity was recorded by custom-made, bipolar electrodes made of silver amalgam (diameter: 4 mm) that were placed over the belly of the right masseter after thorough cleaning of the skin with alcohol. The electrodes were placed parallel to the muscle fibres and with their centres approximately 15

mm apart. The EMG signals were filtered (bandwidth 5-1000Hz), amplified (1000 times), and sampled (2000 Hz). An isolated metal common electrode held in the palm of the hand served as reference electrode. Participants were instructed to keep the EMG activity as constant as possible during the exercise. For the first 20 participants (10 males, 10 females; mean age±SD=26.9±5.2 years) the EMG level was set at 10% of the maximum voluntary EMG activity recorded at baseline; for the other 20 participants (10 males, 10 females; mean age±SD=28.5±9.3 years), it was set at 30%.

Figure 1. Muscle stretcher (Reprinted from Arc Oral Biol 2010;55:621-626, Kemal S. Türker, Michail Koutris, N. Ceren Sümer, E. Sibel Atis, Ian R. Linke, Frank Lobbezoo, Machiel Naeije, Provocation of delayed-onset muscle soreness in the human jaw-closing muscles, with permission from Elsevier).

Collected Data

All data were collected by three experimenters (MK, ESA, and NS), who followed the same data-collection protocol. The clinical examination was performed by two experienced clinicians (MK and FL), who are calibrated on a yearly basis. Before the start of the present study, an extra calibration session was performed.

Fatigue and pain intensity

The subjective feelings of fatigue and pain intensity were measured with two, 100-mm long visual analogue scales (VAS), with left anchor words "No fatigue/pain at all", and right anchor words "Fatigue/pain as bad as it could be". VAS values below 5 mm were set to zero (Ekblom and Hansson, 1988).

Pain-free maximum mouth opening (MMO)

The MMO was recorded while the participants opened their mouth as wide as possible without experiencing any pain. The distance between the incisal edges of the right upper and lower central incisors was measured (in mm) with a plastic ruler and was adjusted for the vertical overbite.

Pressure-pain thresholds (PPTs)

PPTs were recorded from the right and left masseter muscles (RM and LM) and the right and left anterior temporalis muscles (RAT and LAT). A custom-made electronic algometer with a tip of 1 cm in diameter was used. The rate of pressure application was 30 kPa/sec (Svensson et al., 2003), which was visually displayed on a computer screen. While the jaw muscles were relaxed, the experimenter applied the tip of the algometer perpendicular to

the skin at the points of the muscles' maximum bulge. These points were marked before the start of the experiment while the participants clenched several times. For each muscle, four PPT recordings were obtained right after each other, with at least 1 minute rest in between. For the data analysis, the mean values of the last three recordings were used (Isselée et al., 1998).

Jaw-muscle palpation

Twenty orofacial muscle sites were palpated according to the guidelines of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC-TMD) (Dworkin and LeResche, 1992). Following the algorithm of the RDC-TMD, a diagnosis of myofascial pain was set.

Statistical analysis

Because the VAS scores of fatigue and pain intensities were not normally distributed and had unequal variances at different time points, Friedman's ANOVA's were used to test for possible differences in the VAS scores over time. In case of significance, contrast analyses with Wilcoxon signed-rank tests were performed with the values at baseline as reference. Subsequently, for each participant, the medians of the VAS scores over all time points were calculated. Using Mann-Whitney U tests, these medians were tested for gender and intensity level differences.

ANOVAs for repeated measures were used for the analysis of the MMO, the PPTs, and the number of painful muscle-palpation sites. For the MMOs and the number of painful palpation sites, "time" (with 5 levels: before and immediately after the provocation part, and 24 hours, 48 hours, and one week later) was the within-subject factor; and "gender" (with 2 levels: male and female) and "intensity level" (with 2 levels: 10% and 30%) were the between-subjects factors. For the PPTs, an extra within-subject factor was added, viz., "muscle" (with 4 levels: RM, LM, RAT, and LAT). In case of significance, simple contrast analyses were performed, with the values at baseline as reference.

The number of myofascial pain diagnoses, averaged over three time points (immediately, 24 hours, and 48 hours after the provocation part), was calculated for each participant. A possible difference between men and women in the number of positive diagnoses was tested with a Chi-square test. Differences between the two intensity levels were analyzed similarly.

RESULTS

Significant and long-lasting responses to the eccentric and concentric contractions of the jaw muscles were found in all variables investigated (ANOVA's, $p<0.001$). Contrast analysis demonstrated that the fatigue and pain intensity, and the number of painful muscle-palpation sites showed an immediate (immediately after the provocation part) and a delayed (24 hours and 48 hours after the provocation part) increase with respect to baseline; see figure 2A and 2B. Significant decreases with respect to baseline were found for the MMOs

(immediate and delayed) (Fig 2C) and for the PPTs (delayed only) (Fig 2D). All variables had returned to baseline after one week.

The temporalis muscles showed higher PPT values than the masseter muscles ($p=0.001$) and men showed higher PPT values than women ($p=0.003$). No gender influence was found for any of the other variables ($p>0,05$ for all variables tested). No influence of the intensity level was found, except for the reported fatigue levels. These levels were higher for the protocol with the 30% intensity level than for the protocol with the 10% intensity level ($p=0.04$) and are thus reported in separate boxplots (Fig 2B).

Based on the above provoked clinical signs and symptoms and following the diagnostic algorithm of RDC-TMD, up to thirty-one (77.5%) participants received a myofascial pain diagnosis immediately, or 1 or 2 days after the provocation part (Fig 2F). No significant differences in the number of myofascial pain diagnoses were found between men and women or between the two feedback levels.

DISCUSSION

In this study, a protocol involving concentric and eccentric contractions of the jaw muscles could provoke a state of DOMS in the muscles of healthy individuals. The accompanying signs and symptoms yielded the temporary diagnosis of myofascial pain according to the Research Diagnostic Criteria for TMD in 77.5% of these individuals.

The present study indicates that intense, unaccustomed eccentric and concentric muscle contractions can induce immediate and delayed symptoms of fatigue and pain in the human jaw muscles. The symptoms present immediately after the provocation part were probably the result of an accumulation of metabolites, such as lactic acid, within the muscles due to an obstruction of the muscles' blood flow during the exercise (Graven-Nielsen et al., 2003; Kim et al., 1999; Mills et al., 1982; Rasmussen et al., 1977). The delayed responses resembled the characteristics of a so-called DOMS in the human jaw muscles. In limb muscles, DOMS typically develops 12-48 hours after an intense, unaccustomed, eccentric exercise (Friden, 2002; Proske, 2005) and includes the characteristics that were also found in the present study, viz., decreased range of motion, pain at rest, pain exacerbating during movement, and the muscle being painful to palpation. It is generally assumed that the symptoms of DOMS arise from micro traumata to the muscle fibres due to mechanical overloading and rupture especially during the eccentric contractions. The repair of muscle damage is associated with an inflammatory reaction within the muscles (Proske, 2005), which is responsible for a peripheral sensitization of the primary afferent nerve fibers (Regueme et al., 2005). In this study, peripheral sensitization was present on the days following the provocation part, as revealed by the lower PPTs and the muscles' tenderness to palpation. The differences in PPT values found between the temporalis and the masseter muscles corroborate previous studies (Ohrbach and Gale, 1989), as do the lower PPTs values found for women (Jensen et al., 1992).

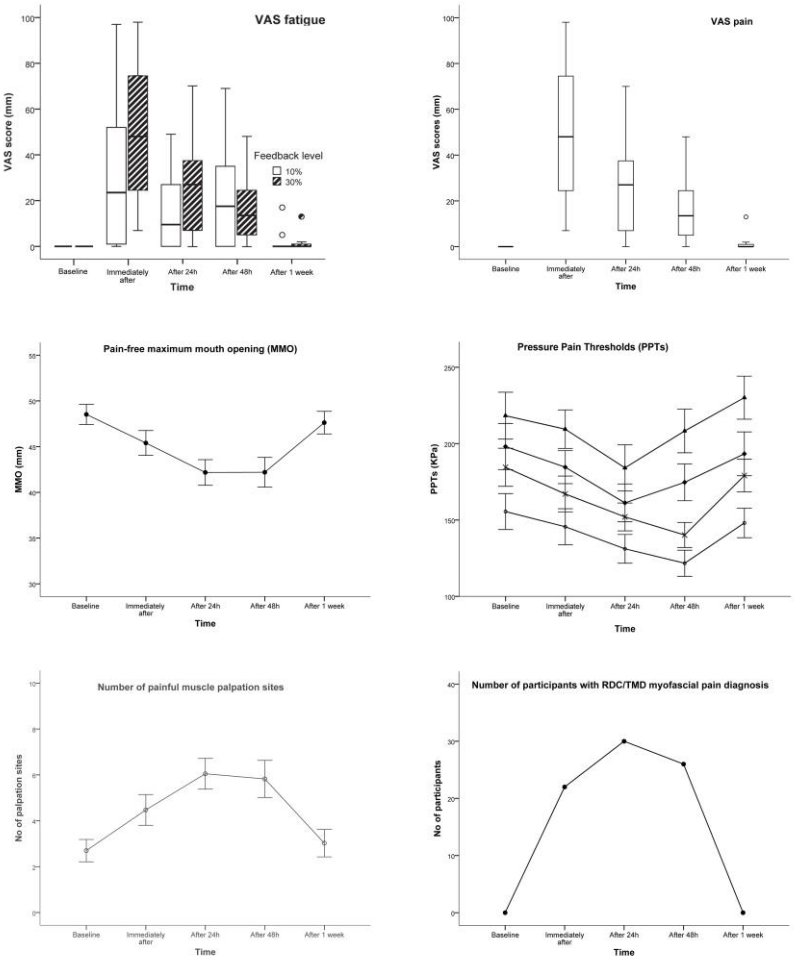

Figure 2. Box plots of the VAS scores of fatigue (for intensity levels at 10% and 30% of the maximum voluntary EMG activity recorded at baseline, since they showed significantly different results) (a) and pain (b), and mean values ± standard errors of the mean of the maximum mouth opening without pain (MMO) (c); the pressure-pain thresholds (PPT) of the masseter (Mas) and the anterior temporalis (Temp) muscles for men (Men) and women (Women) (-♦- Mas_Men, -■- Mas_Women, -▲- Temp_Men, -x- Temp_Women) (d); the number of painful muscle palpation sites (e); and the number of participants with a myofascial pain diagnosis (f). All variables were recorded before and immediately after the provocation part, and after 24 hours, 48 hours, and one week. (*: $p<0.05$; **: $p<0.01$)

The findings of the present study strengthen the suggestion that the myofascial pain in TMD patients may be a manifestation of DOMS in the jaw muscles. In TMD patients associations has been found between self-reported bruxism and TMD pain (Magnusson et al., 2005) and it is well-known that during this parafunctional activity the jaw muscles also perform eccentric contractions (Kato et al., 2003b). In the present study, the myofascial pain was provoked by a single provocation protocol and had subsided after one week indicating that the provoked symptoms were only transient. However, in myofascial TMD patients, the pain complaints are often chronic. This is probably related to the fact that TMD patients usually do not show their bruxing behavior only once in a while, but more on a regular, sometimes even daily basis. It can then be hypothesized that the provoked jaw muscle complaints will run the risk of becoming chronic in case the relaxation periods in between the parafunctional activities are too short for the jaw muscles to recover and to adapt to their overuse. From sports medicine, it is known that when there is an imbalance between training and recovery, exercise and exercise capacity, there is a risk for overtraining the exercised muscles (Lehmann et al., 1993). Thus in TMD patients who brux on a regular basis, the myofascial pain could be the manifestation of a chronic DOMS in their jaw muscles.

The absence of any gender effect in the pain and fatigue scores in this study is in accordance with the results of similar studies in limb muscles (Dannecker, 2008). It may indicate that the higher prevalence of women among jaw muscle-pain patients (Anastassaki and Magnusson, 2004) is not related to a different (patho)physiology of their jaw muscles, which would render these muscles more susceptible to overuse. It may very well be so that the higher prevalence is more related to a higher susceptibility of women to the psychosocial factors, known to be involved in the development of especially chronic pain complaints (Svensson and Sessle, 2004). No standardized experimental protocols exist so far for the provocation of DOMS in skeletal muscles, also not in the human jaw muscles. For this reason two EMG feedback levels were used in this study. More insight into the pathophysiological mechanisms of DOMS is needed to appreciate that the exercise protocol set at a 30% EMG feedback level gives more or less the same results as the feedback level set at 10%.

Though the majority of the participants showed jaw-muscle pain following the provocation protocol, 9 (22.5%) of the participants did not report any pain at all. Apparently, large differences in the jaw muscles' susceptibility to overuse exist. This can also be observed clinically in patients with severe signs of bruxism (e.g., hypertrophic jaw muscles), who sometimes present themselves without any muscle pain, but only with severe tooth wear as their main complaint. This substantiates the claim that there is no simple one-to-one relationship between muscle overuse on one hand and muscle pain on the other hand (Svensson et al., 2008).

Within the limits of the study it was shown that an experimental protocol involving concentric and eccentric muscle contractions can provoke a delayed onset of muscle soreness (DOMS) in the jaw muscles of healthy individuals. The accompanying signs and symptoms yield the temporary diagnosis of myofascial pain according to the RDC-TMD in these individuals. This strengthens the suggestion that the myofascial pain in TMD patients may be a manifestation of DOMS in the jaw muscles.

REFERENCES

Anastassaki A, Magnusson T (2004). Patients referred to a specialist clinic because of suspected temporomandibular disorders: a survey of 3194 patients in respect of diagnoses, treatments, and treatment outcome. *Acta Odontol Scand* 62(4)*:*183-192.

Cheung K, Hume P, Maxwell L (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 33(2)*:*145-164.

Dannecker E (2008). Sex-related differences in delayed-onset muscle pain. In: Fundamentals of Musculoskeletal Pain. T Graven-Nielsen, L Arendt-Nielsen and S Mense editors. Seattle: IASP Press, pp. 249-261.

de Leeuw R (2008). Orofacial Pain. Guidelines for assessment, diagnosis, and management. Chicago: Quintessence Publishing Co, Inc.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6(4)*:*301-355.

Ekblom A, Hansson P (1988). Pain intensity measurements in patients with acute pain receiving afferent stimulation. *J Neurol Neurosurg Psychiatry* 51(4)*:*481-486.

Friden J (2002). Delayed onset muscle soreness. *Scand J Med Sci Sports* 12(6)*:*327-328.

Graven-Nielsen T, Jansson Y, Segerdahl M, Kristensen JD, Mense S, Arendt-Nielsen L *et al*. (2003). Experimental pain by ischaemic contractions compared with pain by intramuscular infusions of adenosine and hypertonic saline. *Eur J Pain* 7(1)*:*93-102.

Isselée H, De Laat A, Bogaerts K, Lysens R (1998). Short-term reproducibility of pressure pain thresholds in masticatory muscles measured with a new algometer. *J Orofac Pain* 12(3)*:*203-209.

Jensen R, Rasmussen BK, Pedersen B, Lous I, Olesen J (1992). Cephalic muscle tenderness and pressure pain threshold in a general population. *Pain* 48(2)*:*197-203.

Kato T, Dal-Fabbro C, Lavigne GJ (2003a). Current knowledge on awake and sleep bruxism: overview. *Alpha Omegan* 96(2)*:*24-32.

Kato T, Thie NM, Huynh N, Miyawaki S, Lavigne GJ (2003b). Topical review: sleep bruxism and the role of peripheral sensory influences. *J Orofac Pain* 17(3)*:*191-213.

Kim YJ, Kuboki T, Tsukiyama Y, Koyano K, Clark GT (1999). Haemodynamic changes in human masseter and temporalis muscles induced by different levels of isometric contraction. *Arch Oral Biol* 44(8)*:*641-650.

Lehmann M, Foster C, Keul J (1993). Overtraining in endurance athletes: a brief review. *Med Sci Sports Exerc* 25(7)*:*854-862.

LeResche L (1997). Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med* 8(3)*:*291-305.

Magnusson T, Egermarki I, Carlsson GE (2005). A prospective investigation over two decades on signs and symptoms of temporomandibular disorders and associated variables. A final summary. *Acta Odontol Scand* 63(2)*:*99-109.

Manfredini D, Guarda-Nardini L, Winocur E, Piccotti F, Ahlberg J, Lobbezoo F (2011). Research diagnostic criteria for temporomandibular disorders: a systematic review of axis I epidemiologic findings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 112(4)*:*453-462.

McNeil C (1993). Temporomandibular Disorders. Second ed. Illinois: Quintessence Publishing Co.

Mills KR, Newham DJ, Edwards RH (1982). Force, contraction frequency and energy metabolism as determinants of ischaemic muscle pain. *Pain* 14(2)*:*149-154.

Ohrbach R, Gale EN (1989). Pressure pain thresholds in normal muscles: reliability, measurement effects, and topographic differences. *Pain* 37(3)*:*257-263.

Proske U (2005). Muscle tenderness from exercise: mechanisms? *J Physiol* 564(Pt 1)*:*1.

Rasmussen OC, Bonde-Petersen F, Christensen LV, Moller E (1977). Blood flow in human mandibular elevators at rest and during controlled biting. *Arch Oral Biol* 22(8-9)*:*539-543.

Regueme SC, Nicol C, Barthelemy J, Grelot L (2005). Acute and delayed neuromuscular adjustments of the triceps surae muscle group to exhaustive stretch-shortening cycle fatigue. *Eur J Appl Physiol* 93(4)*:*398-410.

Svensson P, Arendt-Nielsen L (1995). Effect of topical NSAID on post-exercise jaw muscle soreness: a placebo-controlled experimental study. *J Musculoskel Pain* 3(4)*:*41-58.

Svensson P, Houe L, Arendt-Nielsen L (1997). Effect of systemic versus topical nonsteroidal anti-inflammatory drugs on postexercise jaw-muscle soreness: a placebo-controlled study. *J Orofac Pain* 11(4)*:*353-362.

Svensson P, Cairns BE, Wang K, Hu JW, Graven-Nielsen T, Arendt-Nielsen L *et al.* (2003). Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. *Pain* 101(3)*:*221-227.

Svensson P, Sessle B (2004). Orofacial Pain. In: Clinical Oral Physiology. T Miles, B Nauntofte and P Svensson editors. Copenhagen: Quintessence Publishing Co. Ltd., pp. 93-139.

Svensson P, Jadidi F, Arima T, Baad-Hansen L, Sessle BJ (2008). Relationships between craniofacial pain and bruxism. *J Oral Rehabil* 35(7)*:*524-547.

Türker KS, Koutris M, Sumer NC, Atis ES, Linke IR, Lobbezoo F *et al.* (2010). Provocation of delayed-onset muscle soreness in the human jaw-closing muscles. *Arch Oral Biol* 55(9)*:*621-626.

Torisu T, Wang K, Svensson P, Laat AD, Yamabe Y, Murata H *et al.* (2008). Does eccentric-exercise-induced jaw muscle soreness influence brainstem reflexes? *Clin Neurophysiol* 119(12)*:*2819-2828.

Torisu T, Wang K, Svensson P, Laat AD, Tanaka M, Shimada A *et al.* (2010). Effects of eccentric jaw exercise on temporal summation in jaw-closing muscles of healthy subjects. *Eur J Pain (In press).*

Wijnhoven HA, de Vet HC, Picavet HS (2006). Explaining sex differences in chronic musculoskeletal pain in a general population. *Pain* 124(1-2)*:*158-166.

Chapter 6

Comorbidity negatively influences the outcomes of diagnostic tests for musculoskeletal pain in the orofacial region.

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Pain, 2013, 154: 927-932

ABSTRACT

The aim of this study was to investigate whether diagnostic tests for musculoskeletal pain in the orofacial region (temporomandibular disorder (TMD) pain) are influenced by the presence of comorbid conditions, and to determine whether this influence decreases when the presence of "familiar pain" is used as outcome measure. In total, 117 patients (35 males, 82 females; 75 TMD-pain patients, 42 pain-free patients; mean age±SD=42.94±14.17 years) were examined with palpation tests and dynamic/static tests. After each test, they were asked whether any pain was provoked and whether this pain response was familiar or not. For four clinical outcome measures (pain on palpation, familiar pain on palpation, pain on dynamic/static tests, and familiar pain on dynamic/static tests), multiple logistic regression analyses were performed with the presence of TMD pain as the primary predictor and regional (neck/shoulder) pain, widespread pain, depression, and somatization as comorbid factors. Pain on palpation was not associated with the primary predictor but with regional pain (P=0.02, OR=4.59) and somatization (P=0.011, OR=8.47), whereas familiar pain on palpation was associated with the primary predictor (P=0.003, OR=5.23) but also with widespread pain (P=0.001, OR=2.02). Pain on dynamic/static tests was associated with the primary predictor (P<0.001, OR=11.08) but also with somatization (P=0.037, OR=4.5), whereas familiar pain on dynamic/static tests was only associated with the primary predictor (P<0.001, OR=32.37). In conclusion, diagnostic tests are negatively influenced by the presence of comorbidity. This influence decreases when the presence of familiar pain is used as outcome measure.

Key words: temporomandibular disorders, comorbidity, familiar pain, palpation, dynamic/static tests

ACKNOWLEDGEMENTS

This study has been funded by the Scientific College Physical Therapy (WCF). The authors declare that they have no conflicts of interest.

INTRODUCTION

Musculoskeletal pain, like low-back pain and neck/shoulder pain, is a common complaint that is reported by 8-44% of the general population and has a higher prevalence in women than in men (McBeth and Jones, 2007). It is usually characterized by a dull, aching pain, which aggravates on function. Musculoskeletal pain in the orofacial region is referred to as temporomandibular disorder (TMD) pain (de Leeuw, 2008), and has a point prevalence of approximately 10% (LeResche, 1997). In chronic pain patients, comorbid conditions like other physical symptoms or psychological factors are quite common (Hestbaek et al., 2003; Hoffmann et al., 2011; Velly et al., 2010; Visscher et al., 2001b).

Several classification systems for musculoskeletal pain have been proposed (e.g., (Dworkin and LeResche, 1992; Merksey and Bogduk, 1994; Spitzer et al., 1987; Spitzer and Skovron, 1995)). Among these systems, the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (Dworkin and LeResche, 1992) is unique in its detailed description and standardization of the clinical examination needed for the classification. In the recognition of the various subtypes of TMD pain, i.e. myofascial pain and arthralgia, palpation tests play a crucial role. Also the classification system for the widespread pain condition fibromyalgia is largely based on the outcome of palpation tests (Wolfe et al., 1990). However, in more recent papers, the use of palpation as a diagnostic tool has been challenged (Wolfe, 2003). Critics have indicated that palpation is more an indication of general distress than of muscle pain (Croft, 2000), while others have reported that palpation is merely related to a generalized heightened pain perception (Goldenberg, 1999).

Evidence for the validity of diagnostic tests for musculoskeletal pain is scarce. Recently, two multicenter studies to the validity of the RDC/TMD have been published (Truelove et al., 2010; Visscher et al., 2009). Although the results of these studies are quite contradictory, they agree that the validity of the RDC/TMD is not acceptable. As an alternative to be used in the recognition of musculoskeletal pain, dynamic/static tests have been proposed (Naeije and Hansson, 1986; Visscher et al., 2009). These tests make use of the characteristic of musculoskeletal pain that it aggravates on dynamic or static function (Cyriax and Patricia, 1993).

The above-mentioned validity studies were performed in chronic TMD-pain patients, of which the majority reported physical or psychological comorbidity. It is well known that comorbidity negatively influences treatment outcome (Hoffmann et al., 2011). However, whether comorbidity also influences the outcomes of diagnostic tests is unknown. Ideally, for the recognition of a specific musculoskeletal pain disorder, like TMD pain, the outcomes of the tests should only be related to the presence of that disorder, and not to the presence of comorbidity.

A concept that may further improve the validity of diagnostic tests, is the concept of ‘familiar pain’: only a pain response which resembles the patient’s pain complaints is

interpreted as a positive test outcome (Truelove et al., 2010; Visscher et al., 2009). As compared to the various classification systems (where 'any pain' is interpreted as a positive outcome), it may be expected that the use of familiar pain will lead to a decrease of false positive responses.

The aim of this study was to investigate whether diagnostic tests (palpation and dynamic/static tests) for musculoskeletal pain in the orofacial region are influenced by the presence of comorbid conditions, and to determine whether this influence decreases when the presence of "familiar pain" is used as outcome measure.

MATERIALS AND METHODS

Participants

Participants were all patients who visited the tertiary care clinic of Oral Kinesiology at the Academic Centre for Dentistry in Amsterdam (ACTA) for an intake appointment. Over a period of nine months, all consecutive patients (N = 220), were invited to participate in the study. They were referred to the clinic by their dentist or medical specialist because of orofacial pain, functional complaints of the temporomandibular joints (e.g., joint sounds or movement limitations), and/or tooth wear. So, the sample included patients with an orofacial pain complaint as well as patients without pain complaints. They were all given detailed information about the protocol. 77 of the 220 patients denied participation in the study, most often because of time constraints. Furthermore, patients reporting orofacial pain with characteristics compatible with dental pain (N=7) or neuropathic pain (N=4), or patients with incomplete records (N=15), were excluded. Dental pain was considered present when the patient complained of toothache provoked or aggravated by cold or warm food, or by chewing. It was confirmed by clinical signs of dental pathology, like the presence of caries or pain provocation on tooth percussion, on temperature test, or on bite tests, or by periapical radiolucencies or radiological signs of caries. Neuropathic pain was recognized when the pain complaints presented like shock-like pain, or burning pain (Treede et al., 2008). Thus, in total, 117 patients (35 males, mean age±SD = 42.9±14.2 years) were included.

Protocol overview

Prior to their intake appointment, as part of the regular procedure of the clinic of Oral Kinesiology of ACTA, all patients received a set of questionnaires that included a general health questionnaire and the depression and somatization subscale of the Dutch version of the Symptom Checklist 90 (SCL-90) (Dworkin and LeResche, 1992; Lobbezoo et al., 2005). After the intake visit, patients who agreed to participate in the study underwent an additional standardized clinical examination and filled in the body drawing of the McGill pain questionnaire (Melzack, 1975).

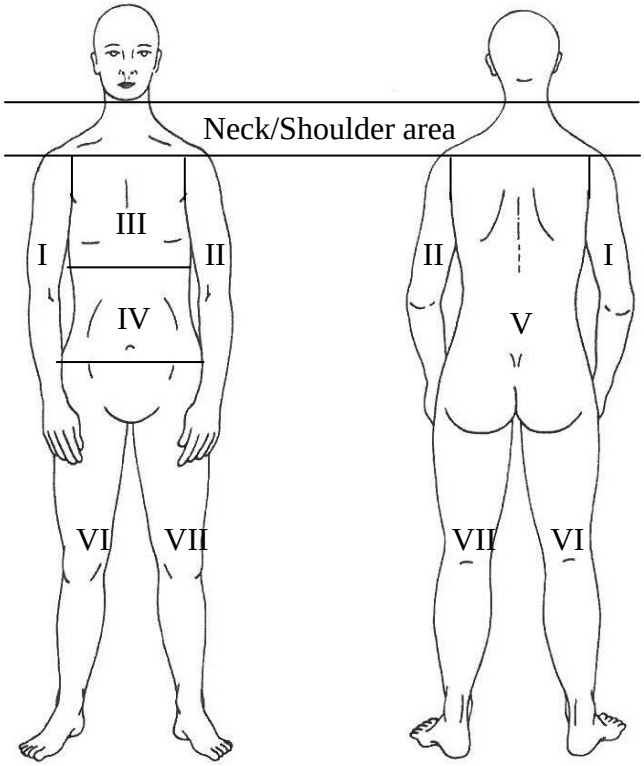

Figure 1. The pain drawing of the McGill pain questionnaire with the body areas' division as used for the analysis of the data.

Standardized clinical examination

The additional standardized clinical examination consisted of palpation tests and dynamic/static tests of the masticatory system. Following each palpation, dynamic, and static test, patients were asked whether or not the test had provoked any pain in the orofacial region. If so, patients were asked whether this pain was familiar or not. The term "familiar pain" was explained to the participants as the pain that resembled their complaints (Truelove et al., 2010; Visscher et al., 2009). The following was asked to the patients: "Do you recognize this pain as (part of) your complaint(s)?" The examination was performed by an examiner who was blind for the presence of orofacial pain complaints and comorbid factors (see below). The examiner was either a thoroughly trained dental student (N=4), or an experienced dentist specialized in TMD patients' management (MK). The students followed a thorough calibration course that included theoretical knowledge about the clinical examination and extensive training of clinical skills under the supervision of a calibrated examiner (MK).

Palpation of the masticatory system

The muscle and joint palpation sites described by the RDC/TMD (Dworkin and LeResche, 1992), with the exception of the intraoral sites and the posterior temporomandibular joint site, were palpated. The intraoral sites and the posterior temporomandibular joint site were excluded because they have a high rate of false positive results (Visscher et al., 2009) and are proposed to be excluded from future classification systems (Schiffman et al.). First, the sites at the left side of the face were examined and then those at the right side. In total, 14 sites were palpated. As specified by the RDC/TMD, a pressure of 2 lbs was applied to the following muscle palpation sites: the posterior, middle, and anterior part of the temporalis muscle, and the origin, body, and insertion of the masseter muscle. A pressure of 1 lb was applied to the posterior mandibular region (corresponding to the stylohyoid/posterior digastric muscles), the submandibular region (corresponding to the medial pterygoid, suprahyoid, anterior digastric muscles), and the lateral pole of the temporomandibular joint. In order to standardize the pressure applied, palpation was performed with the use of a custom-made algometer. The algometer had a stainless-steel handgrip and had a round tip covered with rubber with a diameter of 1 cm. The examiner was holding the algometer perpendicular to the skin above the muscle bulge or the temporomandibular joint. With the use of custom-made software, the examiner received visual feedback on a computer screen while examining the muscles or the temporomandibular joints. The feedback corresponded to a standardized increase of the pressure by 30 kPa/sec until reaching a plateau of 2 lbs or 1 lb, depending on the palpation site. This plateau was kept constant for 3 sec. Then patients were asked whether the test provoked any pain and whether the provoked pain resembled their complaints.

Dynamic/static tests of the masticatory system

The dynamic and static tests (Naeije and Hansson, 1986; Visscher et al., 2007; Visscher et al., 2009) were performed in the following movement directions of the mandible: opening, closing, and protrusion. During dynamic tests, patients performed mandibular movements while the examiner applied a slight manual counter pressure to the movement. During static tests, patients were instructed to hold the mandible motionless, while the examiner gradually increased a manual counter pressure, until either the patient or the examiner reached their maximal effort. This effort was then maintained for about 3 seconds (Visscher et al., 2009). Then patients were asked whether the test provoked any pain and whether the provoked pain resembled their complaints.

Primary predictor – TMD pain

To avoid circularity, the presence of TMD pain cannot be established with the use of the diagnostic tests under study. Since other causes of orofacial pain than TMD pain (e.g., dental pain and neuropathic pain) were excluded, patients reporting pain in the orofacial region were classified as having TMD pain (N=75). When there was no report of orofacial pain at all, they were presumed not to have a TMD pain (N=42).

Comorbid factors (other physical and psychological complaints)

Regional (neck/shoulder) pain and widespread pain: The body drawing of the McGill Pain Questionnaire was used to determine the presence of regional (neck/shoulder) pain and widespread pain (Fig. 1.). After excluding the orofacial area, the body drawing was divided in two parts: the neck/shoulder area (corresponding to regional pain) and the rest of the body (corresponding to widespread pain) (Turp et al., 1997; Visscher et al., 2001a). The "neck/shoulder" area was rated as a separate variable (i.e., not incorporated in the "widespread pain" variable), because of the close association between TMD and cervical spine disorders (De Laat et al., 1998; Visscher et al., 2001b). When pain was marked on the neck/shoulder region, patients were classified as having regional pain, otherwise they were classified as not having regional pain. To rate widespread pain, the body area below the neck and shoulders was divided into seven parts, viz., the right arm, the left arm, the chest, the abdomen, the back, the right leg, and the left leg (Lobbezoo et al., 2004). The total number of painful body areas (ranging from 0-7) was used as an indication of the degree of widespread pain (Fig. 1).

Depression and somatization: These measures were scored with the Dutch version of the SCL-90 (Arrindell and Ettema, 1981). The depression scale assesses negative mood and vegetative symptoms of poor functioning, while the somatization scale assesses distress related to bodily symptoms, such as faintness and stomach upset. The shortened somatization scale, excluding the four pain-related questions, was used in order to avoid confounding with the variables assessing TMD pain or other physical pain complaints (van Selms et al., 2009). Each item of the depression and somatization scale is rated on a five-point Likert scale, ranging from 1 (not at all) to 5 (very much). The total score, taking into account the patient's gender, classifies the patient into one of the 7 categories regarding the severity of the depression and somatization (ranging from very low to very high; 1-7) (Arindell and Ettema, 2003). Finally, the outcomes were dichotomized: patients with scores $\geq$6 (representing high and very high levels of depression and somatization, respectively) were classified as depressed/highly somatized. The others were classified as not-depressed/low on somatization, respectively (Derogatis and Cleary, 1977).

Statistical analysis

To study whether the outcomes of the clinical tests (i.e., palpation and dynamic/static tests) 1) were influenced by the presence of comorbidity and 2) were improved by the addition of the term "familiar" to the pain response, multiple logistic regression models were built for four outcome measures:

- pain on palpation tests
- familiar pain on palpation tests
- pain on dynamic/static tests
- familiar pain on dynamic/static tests

When the patient indicated that orofacial pain was provoked by at least one of the 14 palpation tests, the outcome of the variable 'pain on palpation' was considered positive (scored as 1). When at least one of these pain responses on palpation was recognized as a familiar pain, the variable 'familiar pain on palpation' was considered positive (scored as 1). In all other cases, 'pain on palpation' and 'familiar pain on palpation' were considered absent (scored as 0). Similar rules applied for the classification of 'pain on dynamic/static tests' and 'familiar pain on dynamic/static tests'.

For each outcome variable, first its associations with the primary predictor (TMD pain) and with the comorbid factors [i.e., regional (neck/shoulder) pain, widespread pain, depression, and somatization] were assessed by single logistic regression analyses. Variables that showed at least a moderate association with the outcome variable (i.e., p-value ≤ 0.10) were entered in the multiple regression analysis. Then, the factor with the weakest, non-significant association with the outcome variable was removed from the multiple regression model. This was repeated in a backward stepwise manner until all variables that were retained in the model showed a p-value ≤ 0.05. All regression models (single and multiple) were corrected for age and gender. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated in all analyses.

RESULTS

Table 1 shows descriptive data of the responses to the clinical tests and the outcomes of the primary predictor (TMD pain) and the comorbid factors (regional pain, widespread pain, somatization, and depression). Results of the single and multiple regression analyses are presented in Tables 2 to 5, and are also illustrated in Fig. 2.

Single regression models suggested that "pain on palpation" was not only associated with the presence of TMD pain but also with all of the comorbid factors (regional pain, widespread pain, somatization, and depression). When entered into the multiple regression model, the associations with the primary predictor (TMD pain) and with depression were lost, and only the association with regional pain and somatization was retained in the model (Table 2).

"Familiar pain on palpation" was also associated with all of the predictive factors (single regression analyses). The multiple regression model showed that the association with the primary predictor was retained in the final model, but the association with "widespread pain" remained also present (Table 3).

Single regression models suggested that "pain on dynamic/static tests" was associated with the primary predictor (TMD pain) and the psychological comorbid factors (depression and somatization), but not with the physical comorbid factors (regional and widespread pain). When entered into the multiple regression model, the association with the primary predictor (TMD pain) and also with somatization was retained in the model (Table 4).

"Familiar pain on dynamic/static tests" was also associated with the presence of TMD pain and the psychological comorbid factors (single regression analyses). When entered into the multiple regression analysis though, "familiar pain on dynamic/static tests" was only associated with the primary predictor (TMD pain) and not anymore with any of the comorbid factors (Table 5).

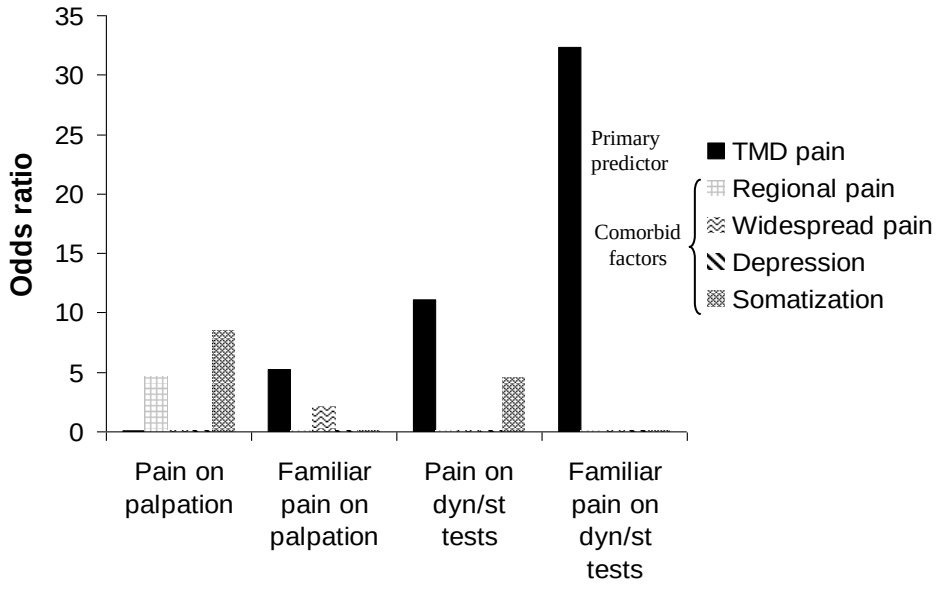

Figure 2. Diagram with the results representing the Odds Ratios of the predictors of the multiple regression analyses for each outcome variable.

Table 1. Descriptives showing the number of participants that responded positively or negatively on the predictive variables (primary predictor and comorbid factors) and on the diagnostic tests. For the comorbid factor "Widespread pain", the median value and the range are reported.

		Palpation tests				**Dynamic/Static tests**			
		Pain		Familiar pain		Pain		Familiar pain	
		No (N=58)	Yes (N=59)	No (N=74)	Yes (N=43)	No (N=63)	Yes (N=54)	No (N=69)	Yes (N=48)
Primary predictor									
TMD pain	No (N=42)	29	13	36	6	37	5	40	2
	Yes (N=75)	29	46	38	37	26	49	29	46
Comorbid factors									
Regional pain	No (N=90)	52	38	64	26	52	38	56	34
	Yes (N=27)	6	21	10	17	11	16	13	14
Depression	No (N=93)	50	43	63	30	54	39	58	35
	Yes (N=24)	8	16	11	13	9	15	11	13
Somatization	No (N=96)	54	42	67	29	58	38	61	35
	Yes (N=21)	4	17	7	14	5	16	8	13
Widespread pain*		0(0-4)	0(0-5)	0(0-4)	0(0-5)	0(0-5)	0(0-5)	0(0-5)	0(0-5)

Table 2. Regression analyses for pain on palpation tests (N=117)

Pain on palpation

	single regression				multiple regression			
	b	p-value	OR	95% CI	b	p-value	OR	95% CI
Primary Predictor								
TMD pain (no/yes)	1.15	**0.01**	3.16	1.32-7.57				
Comorbid factors								
Regional pain (no/yes)	1.97	**0.001**	7.13	2.14-23.81	1.53	**0.02**	4.59	1.28-16.57
Widespread pain (0-7)	0.69	**0.003**	1.99	1.27-3.14				
Depression (no/yes)	1.19	**0.033**	3.29	1.10-9.86				
Somatization (no/yes)	2.56	**0.001**	12.87	2.71-61.14	2.14	**0.011**	8.47	1.63-44.05

The interaction between the variables retained in the multiple regression model was not significant (p-value=0.8). All regression models were corrected for age and gender. Significant results are highlighted with bold letter types (b = regression coefficient, OR = odds ratio; 95% CI = 95% confidence interval).

Table 3. Regression analyses for familiar pain on palpation tests (N=117)

Familiar pain on palpation

	single regression				multiple regression			
	b	p-value	OR	95% CI	b	p-value	OR	95% CI
Primary predictors								
TMD pain (no/yes)	1.7	**0.001**	5.49	1.97-15.29	1.653	**0.003**	5.225	1.76-15.53
Comorbid factors								
Regional pain (no/yes)	1.69	**0.002**	5.46	1.91-15.58				
Widespread pain (0-7)	0.71	**0.001**	2.04	1.35-3.06	0.703	**0.001**	2.02	1.32-3.09
Depression (no/yes)	1.19	**0.025**	3.29	1.16-9.31				
Somatization (no/yes)	2.26	**0.001**	9.55	2.42-37.61				

The interaction between the variables retained in the multiple regression model was not significant (p-value=0.67). All models were corrected for age and gender. Significant results are highlighted with bold letter types (b = regression coefficient, OR = odds ratio; 95% CI = 95% confidence interval).

Table 4. Regression analyses for pain on dynamic/static tests (N=117)

Pain on Dyn/St tests

	single regression				multiple regression			
	b	p-value	OR	95% CI	b	p-value	OR	95% CI
Primary predictor								
TMD pain (no/yes)	2.62	**<0.001**	13.78	4.63-40.96	2.41	**<0.001**	11.08	3.68-33.36
Comorbid factors								
Regional pain (no/yes)	0.72	0.131	2.06	0.81-5.26				
Widespread pain (0-7)	0.21	0.219	1.24	0.88-1.73				
Depression (no/yes)	1.05	**0.042**	2.86	1.04-7.85				
Somatization (no/yes)	1.99	**0.002**	7.36	2.05-26.48	1.51	**0.037**	4.50	1.09-18.57

The interaction between the variables retained at the multiple regression model was not significant (p-value=0.14). All models were corrected for age and gender. Significant results are highlighted with bold letter types (b = regression coefficient, OR = odds ratio; 95% CI = 95% confidence interval).

Table 5: Regression analyses for familiar pain on dynamic/static tests (N=117)

Familiar pain on Dyn/St tests

	single regression				multiple regression			
	b	p-value	OR	95% CI	b	p-value	OR	95% CI
Primary predictor								
TMD pain (no/yes)	3.48	**<0.001**	32.37	7.01-149.53	3.48	**<0.001**	32.37	7.01-149.53
Comorbid factors								
Regional pain (no/yes)	0.59	0.214	1.81	0.71-4.62				
Widespread pain (0-7)	0.21	0.229	1.23	0.88-1.73				
Depression (no/yes)	0.88	**0.084**	2.42	0.89-6.56				
Somatization (no/yes)	1.29	**0.026**	3.64	1.17-11.33				

Significant results are highlighted with bold letter types (b = regression coefficient, OR = odds ratio; 95% CI = 95% confidence interval).

DISCUSSION

To our knowledge, this is the first study that investigated the effects of comorbidity on the outcomes of diagnostic tests for musculoskeletal pain in the orofacial region. The results indicated that the presence of comorbidity influences the outcomes of both palpation and dynamic/static tests, especially when "pain" is used as outcome measure. The use of "familiar pain" reduced the influence of comorbidity for both clinical tests: for palpation, the primary predictor (TMD pain) was now retained in the final multiple regression model, whereas for dynamic/static tests the use of familiar pain even eliminated the influence of comorbid factors.

Several methodological aspects of the study need to be addressed. First of all, different examiners performed the standardized clinical examination. The use of different examiners is a problem when the interexaminer reliability of the clinical tests is poor. For the palpation tests and the dynamic/static tests that were used in this study, the interexaminer reliability of well-trained examiners, either students or calibrated examiners, has been found to be fair-to-good to excellent (Dworkin et al., 1990; Leher et al., 2005; Look et al., 2010; Visscher et al., 2007). Since all examiners received intensive training before participating in the clinical examination, the reliability is expected to be at least fair-to-good also.

Second, the recognition of TMD pain was mainly based on exclusion of other causes for orofacial pain. Usually, the presence of TMD pain is based on the results of an oral history and a clinical examination of the masticatory system. However, to avoid circularity (Cohen and Quintner, 1993), the results of the clinical examination could not be used for the recognition of TMD pain. The most prevalent cause of pain in the orofacial region is dental pain (de Leeuw, 2008), which was objectified and excluded by the patients' pain description and a thorough dental examination. The presence of neuropathic pain was excluded according to the criteria of the Special Interest Group on Neuropathic Pain of the International Association for the Study of Pain (Treede et al., 2008). Other causes of orofacial pain, like vascular pain or referred pain from the neck/shoulder area, are rare causes of pain in the patient population referred to our clinic. Hence, it is likely that most, if not all, pain patients included in the study were indeed suffering from TMD pain.

A clinical test for the recognition of musculoskeletal pain should only be associated with the patients' local musculoskeletal pain complaint (TMD pain) and not with comorbid factors. Our findings indicated that comorbidity influences both the outcomes of palpation and of dynamic/static tests for the recognition of TMD pain. For example, the regression analyses showed that pain on palpation is mostly related to the presence of neck/shoulder pain and somatization. Previous studies have also indicated that the validity of palpation (Truelove et al., 2010) and of dynamic/static tests (with the presence of pain as outcome measure) is insufficient (Visscher et al., 2009). In those study populations, however, comorbidity was not taken into consideration, which might explain the

insufficient validity of the clinical tests. The current findings indicated that the use of the presence of familiar pain as outcome measure, reduced the influence of comorbidity on both palpation and dynamic/static tests. "Familiar pain" has only recently been introduced in the scientific orofacial pain literature (Staud, 2009; Visscher et al., 2009). When familiar pain was used, the outcomes of dynamic/static tests were only associated with the presence of TMD pain and not with any of the comorbid factors. Even with the presence of familiar pain as the outcome measure, palpation was still also associated with widespread pain. This may confirm the assumption that palpation is more related to a generalized hypersensitivity (Diatchenko et al., 2006; Treede et al., 2002) than to local musculoskeletal complaints. Hypersensitivity is caused by complex neurophysiological mechanisms characterized by a heightened pain sensitivity and/or reduced pain inhibition (Staud, 2009). This results in decreased pain thresholds (Staud, 2009) and may lead to an increased pain reaction to clinical tests, like palpation.

The positive effect of the use of "familiar pain" as outcome measure in the clinical tests for the recognition of musculoskeletal pain in the orofacial region, suggests that it might probably improve diagnostic tests for other musculoskeletal pains as well, like low-back pain, or even other kinds of pain. This should be taken into account when patients with comorbid factors are evaluated for musculoskeletal complaints. The use of familiar pain may prevent over-diagnosis and unnecessary treatment.

In conclusion, diagnostic tests for musculoskeletal pain in the orofacial region are negatively influenced by the presence of comorbidity. This influence is decreased when the presence of familiar pain is used as outcome measure.

REFERENCES

Arindell W, Ettema J (2003). SCL-90, Handleiding bij een Multidimensionele Psychopathologie-indicator Lisse, The Netherlands.

Arrindell W, Ettema H (1981). Dimensionele structuur, betrouwbaarheid en validiteit van de Nederlandse bewerking van de Symptom Checklist (SCL-90); gegevens gebaseerd op een fobische en een 'normale' populatie. *Nederlands Tijdschrift voor de Psychologie* 36(77-108.

Cohen ML, Quintner JL (1993). Fibromyalgia syndrome, a problem of tautology. *Lancet* 342(8876):906-909.

Croft P (2000). Testing for tenderness: what's the point? *J Rheumatol* 27(11):2531-2533.

Cyriax JH, Patricia C (1993). Cyriax's illustrated manual of orthopaedic medicine.

De Laat A, Meuleman H, Stevens A, Verbeke G (1998). Correlation between cervical spine and temporomandibular disorders. *Clin Oral Investig* 2(2):54-57.

de Leeuw R (2008). Orofacial Pain. Guidelines for assessment, diagnosis, and management Chicago: Quintessence Publishing Co, Inc.

Derogatis LR, Cleary PA (1977). Factorial invariance across gender for the primary symptom dimensions of the SCL-90. *Br J Soc Clin Psychol* 16(4):347-356.

Diatchenko L, Nackley AG, Slade GD, Fillingim RB, Maixner W (2006). Idiopathic pain disorders--pathways of vulnerability. *Pain* 123(3):226-230.

Dworkin SF, LeResche L, DeRouen T, Von Korff M (1990). Assessing clinical signs of temporomandibular disorders: reliability of clinical examiners. *J Prosthet Dent* 63(5):574-579.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6(4):301-355.

Goldenberg DL (1999). Fibromyalgia syndrome a decade later: what have we learned? *Arch Intern Med* 159(8):777-785.

Hestbaek L, Leboeuf-Yde C, Manniche C (2003). Is low back pain part of a general health pattern or is it a separate and distinctive entity? A critical literature review of comorbidity with low back pain. *J Manipulative Physiol Ther* 26(4):243-252.

Hoffmann RG, Kotchen JM, Kotchen TA, Cowley T, Dasgupta M, Cowley AW, Jr. (2011). Temporomandibular disorders and associated clinical comorbidities. *Clin J Pain* 27(3):268-274.

Leher A, Graf K, PhoDuc JM, Rammelsberg P (2005). Is there a difference in the reliable measurement of temporomandibular disorder signs between experienced and inexperienced examiners? *J Orofac Pain* 19(1):58-64.

LeResche L (1997). Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med* 8(3):291-305.

Lobbezoo F, Visscher CM, Naeije M (2004). Impaired health status, sleep disorders, and pain in the craniomandibular and cervical spinal regions. *Eur J Pain* 8(1)*:*23-30.

Lobbezoo F, van Selms MK, John MT, Huggins K, Ohrbach R, Visscher CM *et al.* (2005). Use of the Research Diagnostic Criteria for Temporomandibular Disorders for multinational research: translation efforts and reliability assessments in The Netherlands. *J Orofac Pain* 19(4)*:*301-308.

Look JO, John MT, Tai F, Huggins KH, Lenton PA, Truelove EL *et al.* (2010). The Research Diagnostic Criteria For Temporomandibular Disorders. II: reliability of Axis I diagnoses and selected clinical measures. *J Orofac Pain* 24(1)*:*25-34.

McBeth J, Jones K (2007). Epidemiology of chronic musculoskeletal pain. *Best Pract Res Clin Rheumatol* 21(3)*:*403-425.

Melzack R (1975). The McGill Pain Questionnaire: major properties and scoring methods. *Pain* 1(3)*:*277-299.

Merksey H, Bogduk N (1994). Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms: IASP PRESS.

Naeije M, Hansson TL (1986). Electromyographic screening of myogenous and arthrogenous TMJ dysfunction patients. *J Oral Rehabil* 13(5)*:*433-441.

Schiffman EL, Ohrbach R, Truelove EL, Tai F, Anderson GC, Pan W *et al.* The Research Diagnostic Criteria for Temporomandibular Disorders. V: methods used to establish and validate revised Axis I diagnostic algorithms. *J Orofac Pain* 24(1)*:*63-78.

Spitzer W, LeBlanc F, Dupuis M (1987). Scientific approach to the assessment and management of activity-related spinal disorders. A monograph for clinicians report of the Quebec task force on spinal disorders.

Spitzer W, Skovron M (1995). Scientific monograph of the Quebec task force on whiplash-associated disorders: Rdefiing "whiplash"and its management.

Staud R (2009). Abnormal pain modulation in patients with spatially distributed chronic pain: fibromyalgia. *Rheum Dis Clin North Am* 35(2)*:*263-274.

Treede RD, Rolke R, Andrews K, Magerl W (2002). Pain elicited by blunt pressure: neurobiological basis and clinical relevance. *Pain* 98(3)*:*235-240.

Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW *et al.* (2008). Neuropathic pain: redefinition and a grading system for clinical and research purposes. *Neurology* 70(18)*:*1630-1635.

Truelove E, Pan W, Look JO, Mancl LA, Ohrbach RK, Velly AM *et al.* (2010). The Research Diagnostic Criteria for Temporomandibular Disorders. III: validity of Axis I diagnoses. *J Orofac Pain* 24(1)*:*35-47.

Turp JC, Kowalski CJ, Stohler CS (1997). Temporomandibular disorders--pain outside the head and face is rarely acknowledged in the chief complaint. *J Prosthet Dent* 78(6)*:*592-595.

van Selms MK, Lobbezoo F, Naeije M (2009). Time courses of myofascial temporomandibular disorder complaints during a 12-month follow-up period. *J Orofac Pain* 23(4)*:*345-352.

Velly AM, Look JO, Schiffman E, Lenton PA, Kang W, Messner RP *et al*. (2010). The effect of fibromyalgia and widespread pain on the clinically significant temporomandibular muscle and joint pain disorders--a prospective 18-month cohort study. *J Pain* 11(11)*:*1155-1164.

Visscher CM, Lobbezoo F, de Boer W, van der Meulen M, Naeije M (2001a). Psychological distress in chronic craniomandibular and cervical spinal pain patients. *Eur J Oral Sci* 109(3)*:*165-171.

Visscher CM, Lobbezoo F, de Boer W, van der Zaag J, Naeije M (2001b). Prevalence of cervical spinal pain in craniomandibular pain patients. *Eur J Oral Sci* 109(2)*:*76-80.

Visscher CM, Lobbezoo F, Naeije M (2007). A reliability study of dynamic and static pain tests in temporomandibular disorder patients. *J Orofac Pain* 21(1)*:*39-45.

Visscher CM, Naeije M, De Laat A, Michelotti A, Nilner M, Craane B *et al.* (2009). Diagnostic accuracy of temporomandibular disorder pain tests: a multicenter study. *J Orofac Pain* 23(2)*:*108-114.

Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL *et al.* (1990). The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 33(2)*:*160-172.

Wolfe F (2003). Stop using the American College of Rheumatology criteria in the clinic. *J Rheumatol* 30(8)*:*1671-1672.

Chapter 7

General discussion

Causes of masticatory muscle pain

Masticatory muscle pain is one of the subclassifications of temporomandibular disorders (TMD). The causes of temporomandibular disorder (TMD) pain have been a matter of debate for decades. In the middle 30s of the past century, pain in the pre-auricular area was described as a syndrome consisting of various symptoms like pain within and around the ears, tinnitus, impaired hearing, and a burning sensation in the throat and tongue and at the side of the nose (Costen, 1934). Back then, these symptoms were associated with the loss of occlusal support, thereby putting the etiological focus on the patient's dental occlusion and articulation (Costen, 1934). This etiological concept resulted in the management of TMD patients by means of extensive and irreversible rehabilitation of the patient's dental occlusion. Later on, the role of dental occlusion in the pathogenesis of TMD pain was more and more critically evaluated, while at the same time oral parafunctions were introduced as possible causes for the initiation and perpetuation of TMD pain (Schwartz, 1955). Moreover, Schwartz was the first to mention that pain in the orofacial region could also originate from the masticatory muscles. In the following years, the psychophysiological model was proposed for the etiology of TMD pain, mentioning oral parafunctions as the most important factor initiating TMD pain (Laskin, 1969). In this model, psychological factors were proposed to initiate parafunctions, thereby causing pain.

For disease in general, the importance of psychosocial factors was later on recognized in the so-called biopsychosocial model of disease (Engel, 1977), which is still considered to explain the etiology of TMD pain best (Suvinen et al., 2005). In the biological part of the biopsychosocial model, the role of oral parafunctions is considered pivotal in the initiation and perpetuation of muscle pain in the masticatory system (Greene, 2001). Epidemiologic studies have indicated an association between parafunctions (like bruxism) and TMD signs and symptoms (Johansson et al., 2004; Magnusson et al., 2000). In order to understand the underlying mechanisms, several experimental studies have been performed; for a review see (Svensson et al., 2008). In these studies, the protocols used included series of isometric and concentric contractions of the masticatory muscles in an attempt to mimic the muscles' contractions as they occur during parafunctional activities in humans. The results indicated that although it is possible to induce muscle fatigue and pain in the masticatory muscles, these symptoms disappear shortly after the cessation of the exercises. In the clinic, however, patients report longer lasting symptoms that are present for weeks or even months. In limb muscles, unaccustomed exercises can provoke long lasting pain, known as delayed onset muscle soreness (DOMS) (Cheung et al., 2003). Several attempts have failed to provoke similar symptoms in the masticatory muscles (Svensson and Arendt-Nielsen, 1995). A possible explanation for the fast resolution of the provoked symptoms in masticatory muscles is these muscles' unique composition compared to limb muscles: the highly compartmentalized organization of the muscle fibres

makes them indeed fatigue-resistant (van Eijden and Turkawski, 2001). Therefore, the role of oral parafunctions in the initiation and perpetuation of TMD pain is not yet clear.

In **Chapters 2, 4,** and **5** of this thesis, the role of oral parafunctions in the etiology of persistent TMD pain was investigated. Persistent pain is pain that lasts longer than the expected recovery period of the muscle tissue damage. Two different experimental protocols were used in order to mimic human parafunctional activities, both aiming to investigate the possible provocation of signs and symptoms similar to those reported by TMD-pain patients. In **Chapter 2**, participants were asked to chew at high speed on a big bolus of hard chewing gum for 100 minutes in total. This exercise resembled the habit of gum chewing, albeit with larger bolus size and higher velocity than usual. The results indicated that immediately after the provocation part, most patients, but not all, developed signs and symptoms that led to the diagnosis of myofascial pain according to the Research Diagnostic Criteria for TMD (RDC/TMD) (**Chapter 2**). Even though most of the participants had recovered 24 hours after the provocation part, two of them still received the diagnosis of myofascial pain 1 day after the provocation. Clearly, there was considerable variability in the response of the participants to the experimental protocol. Unfortunately, no further data regarding the psychosocial status of the participants in this study were collected that may have given an explanation for this variability.

In **Chapters 4** and **5**, a newly developed exercise protocol was applied to load the masticatory muscles in a way that better resembles the bruxism activities that are frequently reported by TMD-pain patients. This protocol included an eccentric component in the muscle contractions and was successful in provoking a longer lasting TMD diagnosis of myofascial pain according to the RDC/TMD in 70% of the participants. This diagnosis was present for a longer period of time compared to the protocol described in **Chapter 2**, and resembled more the clinical condition of patients with TMD complaints. Since clinically, eccentric contractions can occur during bruxism activities of the masticatory muscles, it supports the suggestion that such contractions play a role in the initiation of TMD pain.

Still, the transition from pain initiation to pain perpetuation is not yet fully understood. In people practising sports, an imbalance between training and recovery (i.e., overtraining) has been shown to result in long lasting muscle pain (Lehmann et al., 1993). Especially repetitive eccentric muscle contractions are considered responsible for micro-trauma to the muscles (Friden and Lieber, 1998), and consequently for the development of DOMS. When eccentric contractions are repeated daily and with incomplete periods of recovery, this could, together with the influence of other factors included in the biopsychosocial model, contribute to the chronification of pain. In the masticatory system, oral parafunctions that occur on a daily basis may thus yield the typical clinical spectrum of signs and symptoms as reported by chronic TMD patients.

Consequences for motor function

Jaw-muscle pain is known to influence muscle function, causing a disordered jaw motor behaviour (Lobbezoo et al., 2006). The exact interaction between pain and muscle function is, however, not yet fully understood. In the past, several theories have been proposed for the interaction between pain and motor function. The first one is known as the "vicious-cycle" or "pain-spasm-pain cycle" theory (Travell et al., 1942). According to this theory, pain is provoked by muscle spasm and afterwards, pain reflexly produces more muscle spasm which leads to a self-perpetuating cycle. Apart from explaining the consequences of pain on muscle function, this theory also tries to explain the etiology of pain. However, experimental studies could not confirm several aspects of this theory, for the example the presence of spasm in chronic pain patients. Therefore, it is questioned whether the "pain-spasm-pain cycle" theory really represents the mechanism present in patients suffering from pain (Roland, 1986). According to the "vicious cycle" theory, muscle spasm is considered the result of muscle overload. Afterwards, in a cascade of spasm-pain-spasm events, pain may perpetuate and eventually become chronic (Travell and Simons, 1983).

In the early 90's, the "pain adaptation" model, was proposed (Lund et al., 1991). In this model, pain causes an increased activity of the antagonistic muscles and simultaneously a decreased activity of the agonistic muscles. The "pain-adaptation" model predicts that in the presence of pain, the mobility of the painful body part is decreased in order to avoid further damage and to allow time for recovery. This can be considered the immediate benefit for the pain patient. However, the fact that the non-affected muscles adjust their activity in order to compensate for the disturbed activity of the affected muscles, can have negative long-term consequences for the health of the individual. This can create secondary pain in the muscles that are not affected at the first instance (Hodges and Tucker, 2011). The "pain-adaptation" model seemed more plausible than the "vicious-cycle" theory, because it only deals with the effects of pain on the motor function of the musculoskeletal system. However, subsequent experimental studies could not confirm the pain adaptation model in full (Hodges and Tucker, 2011) . Therefore, new theories appeared (Hodges and Tucker, 2011; Murray and Peck, 2007), which appreciate the fact that changes in muscle function due to pain are individualized. Depending on the different components of pain (i.e., sensory-discriminative, motivational-affective, and cognitive-evaluative) that are expressed in certain conditions, the modulation of the motor response can be different. These changes aim to minimize pain and maintain homeostasis at the same time (Murray and Peck, 2007)..

Regarding the influence of pain on motor function, the results of this thesis contributed to increasing our insight regarding this topic. The decreased maximum voluntary bite force (MVBF) in the presence of jaw-muscle pain (**Chapter 4**) corroborates the results of previous experimental studies which showed that when pain is present, the MVBF decreases (Wang et al., 2000a). This finding is in line with the "pain-adaptation"

model, whereby the decreased activity of the agonistic muscles is thought to prevent further damage to the masticatory system (Lobbezoo et al., 2006). However, in contrast to other experimental provocation protocols, no changes in the jaw-stretch reflex amplitude were found (**Chapter 2**). The only study corroborating these findings is an experimental protocol that was also based on a brief chewing exercise (van Selms et al., 2005). This contradicts, though, the results of several experimental studies that provoked jaw-muscle pain with injections of painful substances in the masticatory muscles and found a facilitation of the stretch reflex amplitude when pain was present (Cairns et al., 2003; Svensson et al., 2003; Wang et al., 2000b; Wang et al., 2002; Wang et al., 2004). This reflex facilitation was thought to protect the painful muscles according to the "pain adaptation" model. One reason explaining these contradictory results could be the lower intensity of pain provoked with the chewing exercises (**Chapter 2**) as compared with that provoked with injected substance-induced jaw-muscle pain. A more likely explanation, however, could be the fact that the evoked fatigue and pain after chewing exercises occurs more naturally, allowing the masticatory system to better adapt to the gradually increasing pain. Therefore, the stretch-reflex amplitude will probably not change under clinical pain conditions.

Another contribution of the present thesis to the body of knowledge involves the methodology used to analyse the amplitude of the jaw-stretch reflex. In **Chapter 3**, it was proven that the method of normalizing the reflex amplitude with the averaged rectified EMG activity of the 100-ms pre-stimulus period makes the reflex amplitude independent of the location of the electrodes, provided that they are placed along the muscle fibers. This finding is important, because it indicates that if the jaw-stretch reflex amplitude is acquired and analysed according to the technique described in **Chapter 3**, it is a reliable measure, because it is not influenced by the position of the electrodes over the muscle belly. In the studies that dealt with the effects of substance-induced pain on the jaw-stretch reflex, the reflex amplitude was also determined as described in this chapter. (Cairns et al., 2003; Svensson et al., 2003; Wang et al., 2000b; Wang et al., 2002; Wang et al., 2004). Therefore, the reasons for the contradictory results regarding the modulation of the stretch-reflex amplitude after pain provocation should be searched in physiological mechanisms, and not in the methodology of the reflex recording itself.

Consequences for clinical practise

An important part of a TMD patient's evaluation is establishing the correct diagnosis. Currently, several classification systems for the diagnosis of TMD pain exist (Dworkin and LeResche, 1992; Merksey and Bogduk, 1994; Spitzer et al., 1987; Spitzer and Skovron, 1995). The most widely used one is the Research Diagnostic Criteria for TMD (RDC/TMD) (Dworkin and LeResche, 1992). In the RDC/TMD, the diagnosis mostly depends on the reaction to palpation of the masticatory muscles and temporomandibular joints. The role of palpation in the diagnosis of other musculoskeletal disorders and

conditions like fibromyalgia, though, has been criticised (Wolfe, 2003). Two recent, large-scale multicenter studies (Truelove et al., 2010; Visscher et al., 2009) concluded that the validity of the RDC/TMD is not acceptable. Indeed, the results reported in **Chapter 6** indicate that palpation tests are more related to comorbid factors like regional pain and somatization than to the local TMD-pain complaint. As an alternative to palpation, the use of the dynamic/static tests has been proposed (Naeije and Hansson, 1986). Moreover, it was recently proposed that the use of the "familiar pain" concept could further improve the validity of diagnostic tests (i.e., a diagnostic approach that takes into account the fact whether or not patients recognize the provoked pain as their pain complaint). In **Chapter 6**, a study to the diagnostic ability of palpation and of dynamic/static tests is described, incorporating the influence of comorbid factors, like depression and other pain complaints, on the outcome of the clinical tests. The study demonstrated that only the dynamic/static tests, with "familiar pain" as outcome measure, were uniquely related to the presence of local TMD-pain complaints and not influenced by comorbid factors. The outcome of palpation tests was mostly related to comorbid factors; not to the local TMD complaints of the patients. Hence, in patients with a chronic orofacial pain complaint, in whom comorbid factors like widespread pain or depression are regularly present, a positive reaction to palpation does not necessarily indicate the actual presence of a TMD pain. Since the physical examination of the RDC/TMD mainly relies on palpation tests of the masticatory system, this finding has important implications for the interpretation of current studies in the field of TMD. For example, in studies to patients with chronic orofacial pain complaints, the presence of TMD-pain is likely to be overestimated (false positive RDC/TMD diagnoses). When such patients are included in studies to treatment outcome, treatment success may be underestimated. Therefore, future diagnostic systems for musculoskeletal disorders, among which TMD, should incorporate dynamic/static tests, with "familiar pain" as outcome measure.

Concluding remarks

The studies included in this thesis showed that masticatory muscles' overloading can initiate signs and symptoms of TMD pain in healthy individuals (**Chapters 2, 4, and 5**). The provoked pain influenced both sensory and motor functions of the masticatory system, causing a decrease in pain pressure thresholds (**Chapter 2, 5, and 6**) and variable effects on motor control: while the MVBF decreased when pain was present (**Chapter 5**), the reflex amplitude was not affected (**Chapter 2**).

For the recording of the jaw-stretch reflex, a technique based on the normalization of the raw data was used. This technique was proven to provide an objective measure of the reflex amplitude that was not dependent on the location of the electrodes for the reflex recording (**Chapter 3**).

Regarding pain, it is known that it is a multidimensional experience, affecting both the somatic and psychosocial aspects of an individual's life. In order to diagnose TMD pain, assessment of the somatic and psychosocial condition of the patient (Axis I and Axis II, respectively) is therefore mandatory. The findings from the present thesis (**Chapter 6**) indicate that diagnostic tests for TMD are negatively influenced by a patient's comorbidity. This influence decreases when the presence of familiar pain is used as outcome measure of the clinical tests. The only tests that were not influenced by the presence of comorbidity were the dynamic/static tests with the use of familiar pain as their outcome.

The results of the present thesis have contributed to further unravel the factors influencing the diagnosis of musculoskeletal pain. Moreover, it increased the understanding of the causes of musculoskeletal pain and of its consequences on jaw-motor function. It remains for future research to further elucidate the factors influencing the progression of pain from acute to chronic. If these mechanisms will be defined, this could lead to the development of an etiological management of TMD pain.

REFERENCES

Cairns BE, Wang K, Hu JW, Sessle BJ, Arendt-Nielsen L, Svensson P (2003). The effect of glutamate-evoked masseter muscle pain on the human jaw-stretch reflex differs in men and women. *J Orofac Pain* 17(4)*:*317-325.

Cheung K, Hume P, Maxwell L (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 33(2)*:*145-164.

Costen J (1934). A syndrome of ear and sinus symptoms dependent upon disturbed function of the temporomandibular joint. *Ann Otol Rhinol Laryngol* 43(1)*:*1-15.

Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6(4)*:*301-355.

Engel GL (1977). The need for a new medical model: a challenge for biomedicine. *Science* 196(4286)*:*129-136.

Friden J, Lieber RL (1998). Segmental muscle fiber lesions after repetitive eccentric contractions. *Cell Tissue Res* 293(1)*:*165-171.

Greene CS (2001). The etiology of temporomandibular disorders: implications for treatment. *J Orofac Pain* 15(2)*:*93-105; discussion 106-116.

Hodges PW, Tucker K (2011). Moving differently in pain: a new theory to explain the adaptation to pain. *Pain* 152(3 Suppl)*:*S90-98.

Johansson A, Unell L, Carlsson G, Soderfeldt B, Halling A, Widar F (2004). Associations between social and general health factors and symptoms related to temporomandibular disorders and bruxism in a population of 50-year-old subjects. *Acta Odontol Scand* 62(4)*:*231-237.

Laskin DM (1969). Etiology of the pain-dysfunction syndrome. *J Am Dent Assoc* 79(1)*:*147-153.

Lehmann M, Foster C, Keul J (1993). Overtraining in endurance athletes: a brief review. *Med Sci Sports Exerc* 25(7)*:*854-862.

Lobbezoo F, van Selms MK, Naeije M (2006). Masticatory muscle pain and disordered jaw motor behaviour: Literature review over the past decade. *Arch Oral Biol* 51(9)*:*713-720.

Lund JP, Donga R, Widmer CG, Stohler CS (1991). The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. *Can J Physiol Pharmacol* 69(5)*:*683-694.

Magnusson T, Egermark I, Carlsson GE (2000). A longitudinal epidemiologic study of signs and symptoms of temporomandibular disorders from 15 to 35 years of age. *J Orofac Pain* 14(4)*:*310-319.

Merksey H, Bogduk N (1994). Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms: IASP PRESS.

Murray GM, Peck CC (2007). Orofacial pain and jaw muscle activity: a new model. *J Orofac Pain* 21(4)*:*263-278; discussion 279-288.

Naeije M, Hansson TL (1986). Electromyographic screening of myogenous and arthrogenous TMJ dysfunction patients. *J Oral Rehabil* 13(5)*:*433-441.

Roland M (1986). A critical review of the evidence for a pain-spasm-pain cycle in spinal disorders *Clinical Biomechanics* 1(102-109.

Schwartz LL (1955). Pain associated with the temporomandibular joint. *J Am Dent Assoc* 51(4)*:*394-397.

Spitzer W, LeBlanc F, Dupuis M (1987). Scientific approach to the assessment and management of activity-related spinal disorders. A monograph for clinicians report of the Quebec task force on spinal disorders.

Spitzer W, Skovron M (1995). Scientific monograph of the Quebec task force on whiplash-associated disorders: Rdefiing "whiplash"and its management.

Suvinen TI, Reade PC, Kemppainen P, Kononen M, Dworkin SF (2005). Review of aetiological concepts of temporomandibular pain disorders: towards a biopsychosocial model for integration of physical disorder factors with psychological and psychosocial illness impact factors. *Eur J Pain* 9(6)*:*613-633.

Svensson P, Arendt-Nielsen L (1995). Effect of topical NSAID on post-exercise jaw muscle soreness: a placebo-controlled experimental study. *J Musculoskel Pain* 3(4)*:*41-58.

Svensson P, Cairns BE, Wang K, Hu JW, Graven-Nielsen T, Arendt-Nielsen L *et al.* (2003). Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. *Pain* 101(3)*:*221-227.

Svensson P, Jadidi F, Arima T, Baad-Hansen L, Sessle BJ (2008). Relationships between craniofacial pain and bruxism. *J Oral Rehabil* 35(7)*:*524-547.

Travell J, Rinzler S, Herman M (1942). Pain and disability of the shoulder and arm. Treatment by intramuscular infiltration with procaine hydrochloride. *Journal of the American Medical Association* 120)*:*417-422.

Travell JG, Simons DG (1983). Myofascial pain and dysfunction: the trigger point manual. Baltimore: Williams & Wilkins.

Truelove E, Pan W, Look JO, Mancl LA, Ohrbach RK, Velly AM *et al.* (2010). The Research Diagnostic Criteria for Temporomandibular Disorders. III: validity of Axis I diagnoses. *J Orofac Pain* 24(1)*:*35-47.

van Eijden TM, Turkawski SJ (2001). Morphology and physiology of masticatory muscle motor units. *Crit Rev Oral Biol Med* 12(1)*:*76-91.

van Selms MK, Wang K, Lobbezoo F, Svensson P, Arendt-Nielsen L, Naeije M (2005). Effects of masticatory muscle fatigue without and with experimental pain on jaw-stretch reflexes in healthy men and women. *Clin Neurophysiol* 116(6)*:*1415-1423.

Visscher CM, Naeije M, De Laat A, Michelotti A, Nilner M, Craane B *et al.* (2009). Diagnostic accuracy of temporomandibular disorder pain tests: a multicenter study. *J Orofac Pain* 23(2)*:*108-114.

Wang K, Arima T, Arendt-Nielsen L, Svensson P (2000a). EMG-force relationships are influenced by experimental jaw-muscle pain. *J Oral Rehabil* 27(5)*:*394-402.

Wang K, Svensson P, Arendt-Nielsen L (2000b). Effect of tonic muscle pain on short-latency jaw-stretch reflexes in humans. *Pain* 88(2)*:*189-197.

Wang K, Arendt-Nielsen L, Svensson P (2002). Capsaicin-induced muscle pain alters the excitability of the human jaw-stretch reflex. *J Dent Res* 81(9)*:*650-654.

Wang K, Sessle BJ, Svensson P, Arendt-Nielsen L (2004). Glutamate evoked neck and jaw muscle pain facilitate the human jaw stretch reflex. *Clin Neurophysiol* 115(6)*:*1288-1295.

Wolfe F (2003). Stop using the American College of Rheumatology criteria in the clinic. *J Rheumatol* 30(8)*:*1671-1672.

Chapter 8

Summary

Masticatory muscle pain is known as myogenous temporomandibular disorder (TMD) pain. It has a prevalence of approximately 10% in the general population and affects women more than men. It is usually characterized by a dull, aching pain, which aggravates on function. The etiology of TMD pain is still not clear, and multiple factors are considered to play role. Oral parafunctions, like the habit of chewing gum or bruxism (i.e., clenching and/or grinding of the teeth), are thought to play a pivotal role in the initiation and perpetuation of TMD pain, even though this has not yet been verified unequivocally by epidemiological and experimental studies. At the same time, the effects of TMD pain on the masticatory sensory-motor function are not fully understood either. Two models, the vicious cycle model and the pain adaptation model, have been proposed to explain these effects the best, although neither of them is able to fully explain the effects that are present in TMD-pain patients. In everyday clinical practice, for the establishment of a TMD-pain diagnosis, palpation and dynamic/static tests are commonly used, however without having full insight into the factors that influence the responses of these tests. To further clarify the above-outlined gaps in the current knowledge, this thesis includes five studies. Chapters 2, 4, and 5 describe studies on the role of experimental parafunctions in the provocation of TMD pain and on the effects of this pain on masticatory muscle function. Chapter 3 is a methodological paper about the recording of the jaw-stretch reflex (i.e., the short-latency response of the jaw-closing muscles after a sudden stretch), which is an important descriptor of motor function. Chapter 6 deals with the factors influencing the outcome of clinical diagnostic tests for TMD pain.

The aim of the first study of this thesis (Chapter 2) was to assess the effects of an intense chewing exercise on the masticatory sensory-motor system. It has been proven that nociceptive substances, injected into the masseter muscle, induce pain and facilitate the jaw-stretch reflex. In this study, it was hypothesized that intense and prolonged chewing would provoke similar effects. Fourteen men gave their informed consent and agreed to participate in the study. They performed 20 bouts of 5-minute chewing exercises. After each bout, 20 minutes, and 24 hours after the exercise, all participants were examined for signs and symptoms of TMD. In addition, data were collected regarding muscle fatigue and pain, the normalized reflex amplitude from the left masseter muscle, and pressure-pain thresholds. Fatigue and pain scores had increased during the exercise ($P<0.001$), but the reflex amplitude did not ($P=0.123$). Twenty minutes after the exercise, 12 participants showed signs of myofascial pain or arthralgia. Pressure pain thresholds were decreased 20 minutes after the exercises ($P=0.009$) but also 24 hours afterwards ($P=0.049$). Based on the results of this study, it can be concluded the intense and prolonged chewing exercises can induce fatigue, pain, and decreased pressure-pain thresholds in the masticatory muscles, but that it does not influence the jaw-stretch reflex.

In the second study of this thesis (Chapter 3), it was tested whether normalization of the jaw-stretch reflex amplitude with respect to the pre-stimulus electromyographic

(EMG) activity will make the amplitude more independent of the location of the surface electrodes over the masseter muscle. A 5 × 6 electrode grid was used to record the jaw-stretch reflex from 25 sites over the right masseter muscle of fifteen healthy men. Results showed that there was a significant site dependency of the pre-stimulus EMG activity and the reflex amplitude. High cross-correlation coefficients were found between the spatial distribution of mean pre-stimulus EMG activities and reflex amplitude. When the reflex amplitude was normalized with respect to the pre-stimulus EMG activity, however, no site dependency was found. In conclusion, normalization of the jaw-stretch reflex amplitude by the pre-stimulus EMG activity strongly reduces its spatial dependency.

In the third study of this thesis (Chapter 4), it was aimed to construct an apparatus that can impose intense eccentric contractions to the jaw-closing muscles, and to test the hypothesis that eccentric contractions can provoke symptoms of delayed-onset muscle soreness (DOMS) in these muscles. The provocation apparatus consisted of two tungsten arms, connected by a hinge axis on one end. Participants bite with their anterior teeth on biting plates located on the other end. Each time the experimenter gradually released the compression force of the apparatus' rubber tubings, the mouth was forced open, during which the jaw-closing muscles performed an eccentric contraction. Six male participants thus performed sequences of concentric and eccentric contractions of their jaw-closing muscles in six sets of exercises, each lasting 5 minutes, and with one minute of rest in between. Each set consisted of 60 open-close movements. Before and after the exercises, and after 24 hours, 48 hours, and one week, feelings of fatigue and pain, the maximum mouth opening without pain, the muscles' tenderness to palpation, and the maximum voluntary bite force were recorded. After 24 hours and 48 hours, the levels of fatigue and pain were elevated, the maximum mouth opening without pain decreased, and five of the six participants reported tenderness to palpation. The maximum voluntary bite force also decreased after 24 hours. These findings indicate that this novel apparatus is successful in inducing DOMS in the jaw-closing muscles.

In the fourth study of this thesis (Chapter 5), the possible role of overuse of the jaw muscles in the pathogenesis of jaw-muscle pain was studied. A protocol involving concentric and eccentric muscle contractions was used to provoke a state of DOMS in the jaw-closing muscles of healthy individuals. It was tested whether the accompanying signs and symptoms would yield the temporary diagnosis of myofascial pain according to the research diagnostic criteria for temporomandibular disorders (RDC/TMD) in these individuals. Forty participants (mean age ± SD = 27.7 ± 7.5 years) performed six, 5-minute bouts of eccentric and concentric jaw-muscle contractions. Before and immediately after the exercise, and 24 hours, 48 hours, and one week later, self-reported muscle fatigue and pain, pain-free maximum mouth opening, pressure-pain thresholds, and the number of painful jaw-muscle palpation sites were recorded. Significant signs and symptoms of DOMS in the jaw-closing muscles were found, which all had resolved after one week. In 31

(77.5%) of the participants, these signs and symptoms also gave rise to a temporary diagnosis of myofascial pain according to the RDC/TMD. Within the limits of the study, it was shown that an experimental protocol involving concentric and eccentric muscle contractions can provoke DOMS in the jaw-closing muscles and can yield a temporary diagnosis of myofascial pain according to the RDC/TMD. This strengthens the supposition that the myofascial pain in TMD patients may be a manifestation of DOMS in the jaw muscles.

The aim of the last study of this thesis (Chapter 6) was to investigate whether diagnostic tests for TMD pain are influenced by the presence of comorbid conditions, and to determine whether this influence decreases when the presence of "familiar pain" is used as outcome measure. In total, 117 patients (35 males, 82 females; 75 TMD-pain patients, 42 pain-free patients; mean age ± SD = 42.9 ± 14.2 years) were examined with palpation tests and dynamic/static tests. After each test, they were asked whether any pain was provoked and whether this pain response was familiar or not. For four clinical outcome measures (pain on palpation, familiar pain on palpation, pain on dynamic/static tests, and familiar pain on dynamic/static tests), multiple logistic regression analyses were performed, with the presence of TMD pain as the primary predictor and regional (neck/shoulder) pain, widespread pain, depression, and somatization as comorbid factors. Pain on palpation was not associated with the primary predictor but with regional pain ($P=0.02$, $OR=4.6$) and somatization ($P=0.011$, $OR=8.5$), whereas familiar pain on palpation was associated with the primary predictor ($P=0.003$, $OR=5.2$) but also with widespread pain ($P=0.001$, $OR=2.0$). Pain on dynamic/static tests was associated with the primary predictor ($P<0.001$, $OR=11.1$) but also with somatization ($P=0.037$, $OR=4.5$), whereas familiar pain on dynamic/static tests was only associated with the primary predictor ($P<0.001$, $OR=32.4$). In conclusion, diagnostic tests are negatively influenced by the presence of comorbidity. This influence decreases when the presence of familiar pain is used as outcome measure.

Samenvatting

Kauwspierpijn: oorzaken, gevolgen en diagnostiek

Kauwspierpijn wordt ook wel aangeduid als myogene temporomandibulaire disfunctie-(TMD-)pijn. Deze TMD-pijn heeft een prevalentie van ongeveer 10% in de algemene bevolking en komt meer voor bij vrouwen dan bij mannen. De pijn is gewoonlijk dof-zeurend van aard en neemt in intensiteit toe bij orale functies. De etiologie van TMD-pijn is vooralsnog niet geheel duidelijk. Meerdere factoren worden met TMD-pijn in verband gebracht. Van orale parafuncties, zoals kauwgom kauwen en bruxisme (tandenknarsen en/of klemmen), wordt gesuggereerd dat ze een belangrijke rol spelen bij het initiëren en in stand houden van TMD-pijn, hoewel dit nog niet onomstotelijk is aangetoond met epidemiologische en experimentele studies. Bovendien zijn ook de effecten van TMD-pijn op de sensomotoriek van het kauwstelsel ook nog niet volledig opgehelderd. In de literatuur zijn twee modellen beschreven die deze effecten het beste zouden verklaren: het vicieuze cirkelmodel en het pijn-adaptatiemodel. Met geen van beide modellen kunnen echter alle effecten worden verklaard die geobserveerd kunnen worden bij TMD-pijnpatiënten. In de alledaagse klinische praktijk worden ten behoeve van de TMD-pijndiagnsotiek vaak palpatietesten en dynamisch/statische testen gebruikt, hoewel er nog geen volledig inzicht is in de factoren die de responsen van deze testen kunnen beïnvloeden. Om de hierboven beschreven hiaten in de huidige kennis verder op te vullen, zijn in het kader van dit proefschrift een vijftal studies uitgevoerd. In de hoofdstukken 2, 4 en 5 worden studies beschreven waarin de rol van experimentele parafuncties is onderzocht bij het provoceren van TMD-pijn en waarin de effecten van deze pijn op de kauwspierfunctie zijn bepaald. Hoofdstuk 3 beschrijft een methodologische studie naar de registratie van de kaakrekreflex (d.w.z. de snelle respons op een plotselinge rek van de kaaksluitspieren), die een belangrijke maat vormt voor de motorische functie van het kauwstelsel. Tot slot wordt in hoofdstuk 6 een studie beschreven naar factoren die van invloed zouden kunnen zijn op de uitkomsten van klinische diagnostische testen voor TMD-pijn.

Het doel van de eerste studie in dit proefschrift (hoofdstuk 2) was het bepalen van de effecten van een intensieve en verlengde kauwtaak op de sensomotoriek van het kauwstelsel. Voor het injecteren van nociceptieve substanties in de masseterspier is aangetoond, dat de geïnduceerde pijn de kaakrekreflex faciliteert. De hypothese van de studie in hoofdstuk 2 was dat intens en verlengd kauwen dezelfde effecten zou provoceren. Veertien mannen participeerden in deze studie. Zij oefenden gedurende 20 periodes van 5 minuten een intensieve kauwtaak uit. Na ieder periode en 20 minuten en 24 uur na de kauwtaak werden de deelnemers onderzocht op tekenen en symptomen van TMD. Verder werden gegevens verzameld betreffende spiervermoeidheid en pijn, de genormaliseerde reflexamplitude van de linker masseterspier en de drukpijndrempel. Vermoeidheid- en pijnscores namen toe tijdens de kauwtaak ($P<0,001$), terwijl de reflexamplitude onveranderd bleef ($P=0,123$). Twintig minuten na de kauwtaak vertoonden 12 deelnemers

tekenen van myofasciale pijn of arthralgie. De drukpijndrempel was afgenomen na 20 minuten (P=0,009) en na 24 uur (P=0,049). Op grond van deze resultaten kan worden geconcludeerd dat intensief en langdurig kauwen kan leiden tot spiervermoeidheid en pijn en tot een afgenomen drukpijndrempel bij de kauwspieren, maar niet tot een facilitering van de kaakrekreflex.

In de tweede studie van dit proefschrift (hoofdstuk 3) is onderzocht of het normaliseren van de amplitude van de kaakrekreflex ten opzichte van de prestimulus elektromyografische (EMG-)activiteit de amplitude onafhankelijker maakt van de locatie van de oppervlakte-elektrodes over de masseterspier. Daartoe werd een 5 x 6 elektroderooster over de rechter masseterspier van 15 gezonde mannelijke vrijwilligers aangebracht, teneinde de kaakrekreflex van 25 plekken tegelijk te registeren. De resultaten laten zien, dat er een significante afhankelijkheid van registratielocatie is voor zowel het prestimulus EMG-niveau als de reflexamplitude. Er werden hoge kruiscorrelatiecoëfficiënten gevonden tussen de ruimtelijke verdeling van de gemiddelde prestimulus EMG-activiteit en die van de reflexamplitude. Als de amplitude echter werd genormaliseerd ten opzichte van de prestimulus EMG-activiteit, dan verdween de locatie-afhankelijkheid. De conclusie is derhalve dat het normaliseren van de amplitude van de kaakrekreflex ten opzichte van de prestimulus EMG-activiteit de ruimtelijke afhankelijkheid van de amplitude in sterke mate reduceert.

Het doel van de derde studie in dit proefschrift (hoofdstuk 4) was het construeren van een apparaat waarmee de kaaksluitspieren excentrisch kunnen worden belast, teneinde de hypothese te toetsen dat door deze spieren excentrisch te laten contraheren symptomen van vertraagde inspanningsspierpijn (“delayed-onset muscle soreness”; DOMS) kunnen worden geprovoceerd. Het ontwikkelde provocatie-apparaat bestaat uit twee wolfraamarmen, die aan de ene kan aan elkaar zijn verbonden via een scharnier, terwijl de proefpersoon aan het andere uiteinde met de voortanden op twee bijtplaten kan bijten. Tussen beide armen bevindt zich een deel van een stugge rubberen slang, die door de bijtkracht kan worden samengedrukt. Door het geleidelijk wegnemen van de drukkracht op het rubberen slangdeel wordt de mond open geforceerd, waarbij de kaaksluitspieren een excentrische contractie uitvoeren. Zes mannelijke proefpersonen voerden aldus zes sets met concentrische en excentrische contracties uit. Iedere set duurde 5 minuten en er werd 1 minuut gepauzeerd tussen de sets. Iedere set bestond uit 60 open-sluitbewegingen. Vermoeidheid en pijn, de maximale mondopening zonder pijn, spierpalpatiegevoeligheid en de maximale vrijwillige bijtkracht werden voor en na deze oefeningen gemeten, alsmede na 24 uur, 48 uur en 1 week. Na 24 en 48 uur was er sprake van een toename in vermoeidheid en pijn, was de maximale pijnvrije mondopening afgenomen en werd bij 5 van de 6 deelnemers palpatiegevoeligheid gevonden. Ook was de maximale vrijwillige bijtkracht na 24 uur afgenomen. Deze resultaten laten zien, dat dit nieuwe apparaat succesvol is in het provoceren van DOMS in de kaaksluitspieren.

In de vierde studie van dit proefschrift (hoofdstuk 5) werd de mogelijke rol van overbelasting van de kauwspieren in de pathogenese van kauwspierpijn bestudeerd. Daartoe werd een protocol van concentrische en excentrische spiercontracties gebruikt om DOMS te provoceren in de kaaksluitspieren van gezonde individuen. Vervolgens werd bepaald of de geprovoceerde tekenen en symptomen aanleiding gaven tot een diagnose van myofasciale pijn volgens de "Research Diagnostic Criteria" voor TMD (RDC/TMD). Veertig deelnemers (gemiddelde ± SD leeftijd = 27,7 ± 7,5 jaar) oefenden zes, 5 minuten durende sets met concentrische en excentrische contracties uit. De zelf-gerapporteerde spiervermoeidheid en –pijn, de pijnvrije maximale mondopening, de drukpijndrempel en het aantal palpatiepijnlijke spieren werden voor en direct na de oefeningen geregistreerd, alsmede na 24 uur, 48 uur en 1 week. Er werden significante tekenen en symptomen van DOMS gevonden in de kaaksluitspieren, die allemaal na 1 week weer waren verdwenen. Bij 31 (77,5%) van de deelnemers gaven deze tekenen en symptomen ook aanleiding tot een tijdelijke diagnose van myofasciale pijn volgens de RDC/TMD. Dit versterkt het vermoeden dat myofasciale pijn bij TMD-patiënten een manifestatie van DOMS in de kauwspieren betreft.

Het doel van de laatste studie van dit proefschrift (hoofdstuk 6) was om te onderzoeken of diagnostische testen voor TMD-pijn worden beïnvloed door de aanwezigheid van comorbiditeit en om te bepalen of deze mogelijke invloeden afnemen als de aanwezigheid van bekende pijn ("familiar pain") als uitkomstmaat wordt gebruikt. In totaal werden 117 deelnemers (35 mannen, 82 vrouwen; 75 TMD-pijnpatiënten, 42 pijnvrije personen; gemiddelde ± SD leeftijd = 42,9 ± 14,2 jaar) onderzocht met palpatietesten en dynamisch/statische testen. Na iedere test werd hen gevraagd of er pijn was geprovoceerd en of deze pijn bekend was of niet. Voor vier klinische uitkomstmaten (pijn bij palpatie, bekende pijn bij palpatie, pijn bij dynamisch/statische testen en bekende pijn bij dynamisch/statische testen) werd een multipele logistische regressieanalyse uitgevoerd, met de aanwezigheid van TMD-pijn als primaire voorspeller en met regionale (nek-/ schouder-)pijn, wijdverspreide pijn, depressie en somatisatie als comorbide factoren. Pijn bij palpatie was niet geassocieerd met de primaire voorspeller maar wel met regionale pijn (P=0.02, OR=4,6) en somatisatie (P=0,011, OR=8,5), terwijl bekende pijn bij palpatie wel was geassocieerd met de primaire voorspeller (P=0,003, OR=5,2) maar ook met wijdverspreide pijn (P=0.001, OR=2,0). Pijn bij dynamisch/statische testen was eveneens geassocieerd met de primaire voorspeller (P<0,001, OR=11,1) maar ook met somatisatie (P=0,037, OR=4,5), terwijl bekende pijn bij dynamisch/statische testen alleen maar was geassocieerd met de primaire voorspeller (P<0,001, OR=32,4). De conclusie is derhalve dat diagnostische testen negatief worden beïnvloed door de aanwezigheid van comorbide factoren, maar dat deze invloed afneemt als de aanwezigheid van bekende pijn wordt gebruikt als uitkomstmaat.

Περίληψη

Πόνος μασητήριων μυών: αιτίες, συνέπειες και διάγνωση

Ο πόνος των μασητήριων μυών είναι γνωστός ως πόνος μυϊκού τύπου κρανιογναθικής διαταραχής (ΚΓΔ). Η συχνότητά του εκτιμάται περίπου στο 10% του γενικού πληθυσμού και επηρεάζει περισσότερο τις γυναίκες από τους άντρες. Συνήθως χαρακτηρίζεται από έναν βύθιο πόνο που επιδεινώνεται με την λειτουργία. Η αιτιολογία του πόνου από ΚΓΔ δεν είναι ακόμα σαφής και πολλοί παράγοντες θεωρούνται ότι διαδραματίζουν σημαντικό ρόλο. Παραλειτουργικές έξεις, όπως για παράδειγμα το μάσημα τσίχλας ή ο βρυγμός (το σφίξιμο και/ή το τρίξιμο των δοντιών), θεωρούνται ότι παίζουν πρωταγωνιστικό ρόλο στην έναρξη και στην διαιώνιση του πόνου από ΚΓΔ, παρόλο που κάτι τέτοιο δεν έχει αναμφίβολα επιβεβαιωθεί από επιδημιολογικές και πειραματικές μελέτες. Ταυτόχρονα, οι επιπτώσεις του πόνου από ΚΓΔ στην αισθητική και κινητική λειτουργία του στοματογναθικού συστήματος δεν έχουν επίσης κατανοηθεί πλήρως. Δύο μοντέλα, το μοντέλο του «φαύλου κύκλου» από τη μια και το μοντέλο της «προσαρμογής στον πόνο», έχουν προταθεί ότι εξηγούν αυτές τις επιπτώσεις πληρέστερα, παρόλο που κανένα από τα δύο δεν είναι σε θέση να εξηγήσει πλήρως τις επιπτώσεις που παρατηρούνται στους ασθενείς με πόνο από ΚΓΔ. Στην καθημέρα κλινική πράξη, για να τεθεί η διάγνωση του πόνου από ΚΓΔ, η ψηλάφηση των μυών και τα δυναμικά/στατικά τεστ χρησιμοποιούνται συχνά, χωρίς όμως να υπάρχει πλήρης κατανόηση των παραγόντων που επηρρεάζουν τα αποτελέσματα αυτών των διαγνωστικών τεστ. Για να μειωθεί το κενό γνώσης στα συγκεκριμένα θέματα, η παρούσα διδακτορική διατριβή περιλαμβάνει πέντε εργασίες. Τα Κεφάλαια 2, 4 και 5 περιλαμβάνουν εργασίες για το ρόλο της πειραματικής πρόκλησης παραλειτουργικών έξεων στην πρόκληση πόνου από ΚΓΔ και την επίδραση αυτού του πόνου στη λειτουργία των μασητήριων μυών. Το Κεφάλαιο 3 είναι ένα άρθρο μεθοδολογίας για την καταγραφή του διατατικού αντανακλαστικού της κάτω γνάθου (της σύντομης απόκλισης του ανασπώντων την κάτω γνάθο μυών μετά από μία ξαφνική διάταση), το οποίο αποτελεί μία σημαντική παράμετρο περιγραφής της κινητικής λειτουργίας του στοματογναθικού συστήματος.

Ο σκοπός της πρώτης μελέτης αυτής της διατριβής (Κεφάλαιο 2) ήταν να προσδιορίσει την επίδραση μιας σειράς έντονων μασητικών ασκήσεων στην αισθητική-κινητική λειτουργία του μασητικού συστήματος. Έχει αποδειχθεί ότι αλγαισθητικές ουσίες οι οποίες εγχύονται στους μασητήριους μυς, προκαλούν πόνο και ευαισθητοποιούν το μυοτατικό αντανακλαστικό της κάτω γνάθου. Η ερευνητική υπόθεση αυτής της μελέτης ήταν ότι η έντονη και παρατεταμένη μάσηση θα προκαλούσε παρόμοια αποτελέσματα. Δεκατέσσερις άνδρες έδωσαν τη γραπτή τους συγκατάθεση και συμφώνησαν να συμμετέχουν στη μελέτη. Συνολικά εκτέλεσαν 20 σετ μασητικών ασκήσεων διάρκειας 5 λεπτών έκαστο. Μετά από κάθε σετ ασκήσεων και 24 ώρες μετά, όλοι οι συμμετέχοντες εξετάστηκαν για σημεία και συμπτώματα ΚΔΓ. Επίσης, δεδομένα συλλέχθηκαν αναφορικά με την κόπωση και τον πόνο των μασητήριων μυών, την κανονικοποιημένη ένταση του

μυοτατικού αντανακλαστικού της κάτω γνάθου του αριστερού μασητήρα και τον ουδό πόνου. Η ένταση της κόπωσης και του πόνου αυξήθηκαν κατά τη διάρκεια των ασκήσεων (P<0.001), αλλά η ένταση του αντανακλαστικού όχι (P=0.123). Είκοσι λεπτά μετά τις ασκήσεις, 12 συμμετέχοντες εμφάνισαν σημεία μυοπεριτονιακού πόνου ή αρθραλγίας. Ο ουδός πόνου μειώθηκε 20 λεπτά μετά τις ασκήσεις (P=0.009), αλλά και μετά από 24 ώρες (P=0.049). Με βάση τα αποτελέσματα αυτής της μελέτης, μπορούμε να συμπεράνουμε ότι οι έντονες και παρατεταμένες μασητικές ασκήσεις μπορούν να δημιουργήσουν κόπωση, πόνο και μειωμένο ουδό πόνου στους μασητήριους μύες, χωρίς όμως να επηρεάσουν το μυοτατικό αντανακλαστικό της κάτω γνάθου.

Στη δεύτερη μελέτη αυτής της διατριβής (Κεφάλαιο 2), ερευνήθηκε το κατά πόσον η κανονικοποίηση της έντασης του μυοτατικού αντανακλαστικού της κάτω γνάθου με την ηλεκτρομυογραφική (ΗΜΓ) δραστηριότητα προ του ερεθίσματος, θα οδηγούσε σε μη εξάρτηση της έντασης από τη θέση τοποθέτησης των ηλεκτροδίων στο μασητήρα. Ένα δίκτυο 5 × 6 ηλεκτροδίων χρησιμοποιήθηκε για την καταγραφή του μυοτατικού αντανακλαστικού της κάτω γνάθου από 25 θέσεις του δεξιού μασητήρα μυός 15 υγειών ανδρών. Τα αποτελέσματα έδειξαν μια σημαντική τοπογραφική εξάρτηση της ΗΜΓ δραστηριότητας προ του ερεθίσματος και της έντασης του αντανακλαστικού. Υψηλοί συντελεστές συσχέτισης βρέθηκαν μεταξύ της τοπογραφικής κατανομής της μέσης ΗΜΓ δραστηριότητας και της έντασης του αντανακλαστικού. Ωστόσο, όταν έγινε η κανονικοποίηση σε σχέση με την ΗΜΓ προ του ερεθίσματος, δεν βρέθηκε καμιά τοπογραφική εξάρτηση. Συμπερασματικά, η κανονικοποίηση της έντασης του μυοτατικού αντανακλαστικού της κάτω γνάθου με την ΗΜΓ δραστηριότητα προ του ερεθίσματος, μειώνει σημαντικά την τοπογραφική του εξάρτηση.

Στην τρίτη μελέτη αυτής της διατριβής (Κεφάλαιο 4) ο σκοπός ήταν η κατασκευή μιας συσκευής που θα μπορούσε να προκαλέσει έντονες, έκκεντρες συσπάσεις των ανασπώντων την κάτω γνάθο μυών και η διερεύνηση της υπόθεσης ότι οι έκκεντρες συσπάσεις μπορούν να προκαλέσουν συμπτώματα καθυστερημένης κόπωσης μυών (ΚΚΜ) στους μασητήριους μύες. Η συσκευή πρόκλησης αποτελούνταν από δύο μεταλλικούς βραχίονες από βολφράμιο που ήταν ενωμένοι στο ένα άκρο τους. Οι συμμετέχοντες δάγκωναν με τα πρόσθια δόντια τους στην περιοχή δήξης που βρισκόταν στο άλλο άκρο. Σε κάθε κίνηση ο ερευνητής απελευθέρωνε βαθμιαία τη δύναμη συμπίεσης των ελαστικών δακτυλίων της συσκευής οδηγώντας σε διάνοιξη του στόματος, κατά τη διάρκεια της οποίας οι ανασπώντες μύες της κάτω γνάθου συσπώνταν έκκεντρα. Έξι άντρες συμμετέχοντες υποβλήθηκαν σε μια σειρά έξι σετ έκκεντρων και ομόκεντρων συσπάσεων των ανασπώντων την κάτω γνάθο μυών διάρκειας 5 λεπτών έκαστο με ένα λεπτό ξεκούρασης στο ενδιάμεσο. Κάθε σετ αποτελούνταν από 60 νικήσεις κατάσπασης-ανάσπασης. Πριν και μετά της ασκήσεις, αλλά και 24 ώρες, 48 ώρες και μία εβδομάδα μετά, δεδομένα συλλέχθηκαν για την κόπωση, τον πόνο, τη μέγιστη κατάσπαση της κάτω γνάθου χωρίς πόνο, την ευαισθησία των μυών στην ψηλάφηση και τη μέγιστη οικειοθελή

δύναμη δήξης. 24 και 48 ώρες μετά, τα επίπεδα κόπωσης και πόνου ήταν αυξημένα, η μέγιστη κατάσπαση της κάτω γνάθου χωρίς πόνο ήταν περιορισμένη και πέντε από τους έξι συμμετέχοντες ανέφεραν ευαισθησία στην ψηλάφηση. Η μέγιστη οικειοθελής δύναμη δήξης ήταν επίσης μειωμένη μετά από 24 ώρες. Τα αποτελέσματα αυτά δείχνουν ότι η πρωτότυπη αυτή συσκευή μπορεί να προκαλέσει ΚΚΜ στους ανασπώντες την κάτω γνάθο μυς.

Στην τέταρτη μελέτη αυτής της διατριβής (Κεφάλαιο 5) μελετήθηκε ο πιθανός ρόλος της υπερχρήσης των μασητήριων μυών στην παθογένεση του πόνου από τους μασητήριους μύες. Ένα πειραματικό πρωτόκολλο που περιελάμβανε έκκεντρες και σύγκεντρες συσπάσεις των μυών, χρησιμοποιήθηκε για την πρόκληση μιας κατάστασης ΚΚΜ στους ανασπώντες την κάτω γνάθο μυς υγειών συμμετεχόντων. Ερευνήθηκε το κατά πόσο τα συνοδά σημεία και συμπτώματα θα οδηγούσαν στην πρόσκαιρη διάγνωση μυοπεριτονιακού πόνου, σύμφωνα με τα διεθνή διαγνωστικά κριτήρια έρευνας των κρανιογναθικών διαταραχών (ΔΔΚΕ/ΚΓΔ) στους συμμετέχοντες. Σαράντα συμμετέχοντες (μέση ηλικία ± ΣΑ = 27.7 ± 7.5 έτη) εκτέλεσαν έξι σετ, 5-λεπτών έκαστο έκκεντρων και ομόκεντρων συσπάσεων των μασητήριων μυών. Πριν και αμέσως μετά τις ασκήσεις, αλλά και 24 ώρες, 48 ώρες και μία εβδομάδα αργότερα, δεδομένα συλλέχθηκαν για την κόπωση και τον πόνο των μασητήριων μυών, τη μέγιστη διάνοιξη της κάτω γνάθου χωρίς πόνο, τον ουδό του πόνου και τον αριθμό των επώδυνων σημείων ψηλάφησης των μασητήριων μυών. Σημαντικά σημεία και συμπτώματα ΚΚΜ βρέθηκαν στους ανασπώντες την κάτω γνάθο μυς τα οποία όλα υποχώρησαν μετά από μία εβδομάδα. Σε 31 (77.5%) συμμετέχοντες, αυτά τα σημεία και συμπτώματα οδήγησαν και σε προσωρινή διάγνωση μυοπεριτονιακού πόνου σύμφωνα με τα ΔΔΚΕ/ΚΓΔ. Με τους περιορισμούς αυτής της μελέτης, αποδείχθηκε ότι ένα πειραματικό πρωτόκολλο που περιλαμβάνει ομόκεντρες και έκκεντρες συσπάσεις των μασητήριων μυών μπορεί να προκαλέσει ΚΚΜ στους ανασπώντες την κάτω γνάθο μυς και να οδηγήσει σε προσωρινή διάγνωση μυοπεριτονιακού πόνου σύμφωνα με τα ΔΔΚΕ/ΚΓΔ. Αυτό το γεγονός ισχυροποιεί την υπόθεση ότι ο μυοπεριτονιακός πόνος σε ασθενής με ΚΓΔ αποτελεί μία σκδήλωση ΚΚΜ στους μασητήριους μυς.

Ο σκοπός της τελευταίας έρευνας αυτής της διατριβής (Κεφάλαιο 6) ήταν η διερεύνηση του κατά πόσο τα διαγνωστικά τεστ για τον πόνο από ΚΓΔ επηρεάζονται από καταστάσεις συννοσηρότητας και να προσδιορίσει αν αυτή η επίδραση μειώνεται από τη χρήση του όρου «οικείος πόνος» ως μεταβλητή. Συνολικά, 17 ασθενείς (35 άνδρες, 82 γυναίκες• 75 ασθενείς με ΚΓΔ πόνο, 42 ασθενείς ελεύθεροι πόνου, μέση ηλικία ± ΣΑ = 42.9 ± 14.2 έτη) εξετάστηκαν με ψηλάφηση και με δυναμικά/στατικά τεστ. Μετά από κάθε τεστ, ρωτήθηκαν αν προκλήθηκε πόνος και αν αυτός ο πόνος ήταν οικείος ή όχι. Για τέσσερις μεταβλητές (πόνος στην ψηλάφηση, οικείος πόνος στην ψηλάφηση, πόνος στα δυναμικά/στατικά τεστ και οικείος πόνος στα δυναμικά/στατικά τεστ), χρησιμοποιήθηκαν πολλαπλές αναλύσεις λογιστικής παλινδρόμησης με βασικό προγνωστικό παράγοντα την ύπαρξη ΚΓΔ πόνου και παράγοντες συννοσηρότητας τον περιοχικό (σε λαιμό/αυχένα)

πόνο, τον ευρέως διαδεδομένο πόνο, την κατάθλιψη και τη σωματοποίηση. Ο πόνος στην ψηλάφηση δε συνδεόταν με τον βασικό προγνωστικό παράγοντα, αλλά με τον περιοχικό πόνο (P=0.02, OR=4.6) και τη σωματοποίηση (P=0.011, OR=8.5), ενώ ο οικείος πόνος στην ψηλάφηση συνδεόταν με τον βασικό προγνωστικό παράγοντα (P=0.003, OR=5.2), αλλά και με τον ευρέως διαδεδομένο πόνο (P=0.001, OR=2.0). Ο πόνος στα δυναμικά/στατικά τεστ συνδεόταν με τον βασικό προγνωστικό παράγοντα (P<0.001, OR=11.1), αλλά και με τη σωματοποίηση (P=0.037, OR=4.5), ενώ ο οικείος πόνος στα δυναμικά/στατικά τεστ συνδεόταν μόνο με τον βασικό προγνωστικό παράγοντα (P<0.001, OR=32.4). Συμπερασματικά, τα διαγνωστικά τεστ επηρεάζονται αρνητικά από την ύπαρξη συννοσηρότητας. Αυτή η επίδραση μειώνεται, όταν η παρουσία οικείου πόνου χρησιμοποιείται ως μεταβλητή.

Curriculum vitae

Michail Koutris was born on April 19th, 1979 in Rodos, Greece. In 1997 he started his undergraduate studies in dentistry at the Dental School of the National and Kapodistrian University of Athens where he received his DDS degree on February 2003. In 2004 he moved to the Netherlands to enroll in the advanced master program in Oral Kinesiology at the Department of Oral Function and Restorative Dentistry of the Academic Centre for Dentistry Amsterdam (ACTA). On April 2005 he received a "Marie Currie Training Sites" fellowship and moved for 4 months to the Center for Sensory-Motor interaction in Aalborg, Denmark. He received his advanced master degree in Oral Kinesiology on June 2007. Until July 2010, he worked as a full-time PhD student at ACTA working at the same time as a dentist specialized in Orofacial pain and dysfunction at the referral practice for Endodontology and Gnathology in Hoorn, the Netherlands. From August until May 2010 he served his military service as a dentist at the military hospital of Rodos and at the Hellenic Army headquarters, in Athens, Greece. Michail is, since December 2011, working part-time as an assistant professor at ACTA working on further research projects, participating in patient care, and supporting the advanced master program in Oral Kinesiology. From March 2013 he is also working as an Orofacial pain and dysfunction specialist at the Hospital Dentistry clinic of the department of Oral and Maxillofacial Surgery of the Academic Medical Centre (AMC) in Amsterdam. In Greece, where he currently spends 2 weeks per month, Michail is working as a freelance orofacial pain and dysfunction specialist and as a scientific collaborator of the Orofacial Pain clinic of the Dental School of the National and Kapodistrian University of Athens. Michail is currently chairing the Research Committee of the European Academy of Craniomandibular Disorders (EACD) and is also the national representative of Greece at EACD. He is a full member of the Society of Oral Physiology (Store Kro Club), the International Association for the Study of Pain (IASP), the Dutch Society for Gnathology and Prosthetic Dentistry (NVGPT), and the Greek Society of Orofacial Pain.

List of publications

Publications in peer-reviewed journals

- M Koutris, CM Visscher, F Lobbezoo, M Naeije. Comorbidity negatively influences the outcomes of diagnostic tests for musculoskeletal pain in the orofacial region. Pain 2013; 154: 927-932.
- M Koutris, F Lobbezoo, NC Sümer, ES Atís, KS Türker, M Naeije. Is Myofascial Pain in Temporomandibular Disorder Patients a Manifestation of Delayed-onset Muscle Soreness? Clin J Pain. 2013;29(8):712-716.
- KS Türker, M Koutris, NC Sümer, ES Atís, IR Linke, F Lobbezoo, M Naeije. Provocation of delayed-onset muscle soreness in the human jaw-closing muscles. Arch Oral Biol. 2010; 55(9):621-626.
- M Koutris, M Naeije, F Lobbezoo, K Wang, L Arendt-Nielsen, P Svensson, D Farina. Normalization reduces the spatial dependency of the jaw-stretch reflex activity in the human masseter muscle. Muscle Nerve 2010;41(1):78-84.
- M Koutris, F Lobbezoo, M Naeije, K Wang, P Svensson, L Arendt-Nielsen, D Farina. Effects of Intense Chewing Exercises on the Masticatory Sensory-Motor System. J Dent Res 2009;88(7):658-662.
- P Wetselaar, F Lobbezoo, M Koutris, C Visscher, M Naeije. Reliability of an occlusal and non-occlusal tooth wear grading system: clinical use versus dental cast assessment. Int J Prosthodont 2009;22(4):388-390.

Other publications

- M Koutris, F Lobbezoo. Myofasciale pijn bij temporomandibulaire disfunctie: een manifestatie van vertraagde inspanningsspierpijn (DOMS)? Quality Practice Tandheelkunde, 2013, 8(4): 16-19.
- M Koutris Myogenous temporomandibular disorders. Odontostomatologiki Proodos 2011, 65 (2):210-216. [Article in Greek]
- M Koutris, P Wetselaar, C Papayianni. Systemic Lupus Erythematosus, Sjögren Syndrome, and Reiter Syndrome in the TMD/ Orofacial Pain clinic. NVGPT Bulletin 2007;25(2):6-9.
- P Wetselaar, S Kalaykova, C Papayianni, M Koutris. Primary headaches. NVGPT Bulletin 2007;25(1):18-27.
- M Koutris and P Wetselaar, What are the effects of occlusal forces on periodontal tissues? Published in NVGPT infobulletin, 24e jaargang 2006, druk 1, page 6-7
- S Kalaykova, M Koutris and P Wetselaar, Neuropathic Trigeminal Pain: summary of the Journal of Orofacial Pain, Vol. 18, No 4, Fall 2004. Published in NVGPT infobulletin, 23e jaargang 2005, druk 2, page 26-27

Acknowledgements / Dankwoord / Ευχαριστίες

Athens, September, 2013

One of the most important things that I realized during the years of preparation of this thesis is that putting my thoughts in paper is a demanding and difficult task. And now, at this end, here comes a more challenging task: to put my emotions and my gratefulness in words! And there are many people that made the dream of completing this thesis come true.

Dear professor Naeije, Beste Chiel, your critical approach to every thought / plan / word / sentence / paragraph / discussion have allowed me to totally change the way of approaching both research and clinical practice. I am sincerely grateful for all the time and effort you invested in making me mature as a researcher and clinician. And even though I admit that I often had difficult times during and after our discussion, it was almost always the case that I thought: “You were right!” And “Why did I not think that aspect before?”

Dear professor Lobbezoo, Beste Frank, from your first reply to my e-mail expressing my interest to come to Amsterdam for the advanced master program in Oral Kinesiology (back in July 2004) until our current close collaboration, it has been an enthusiastic experience working together with you. Thank you for giving me the opportunity to study at OKI, to learn from you, and to work in a fine group. Special thanks for encouraging and helping me with international collaborations and projects, but also supporting me in nice but also difficult professional and personal moments.

Dear dr. Visscher, Beste Corine, our close collaboration during the last project of this thesis made me appreciate your hard work and your skills in both statistics and critical thinking and writing. You had the ability to read my thoughts before expressing them in words. This made our meetings and discussions extremely fascinating with new ideas coming up. Thanks a lot for these moments!

Dear dr. van der Zaag, Beste Jacques, thanks to you I had the opportunity to improve my clinical skills at OKI. Our discussions inside and outside ACTA meant a lot for my current and future decisions in life and science.

Dear dr. van Selms, Beste Maurits, you have been the one giving me plenty of advice when I started the first project of this thesis. I will never forget all your practical details regarding procedures, practical issues, and protocol preparations. You have always been present when a question came up, when an extra statistical test was needed, when we both had to deal with longitudinal data… But above all, I would like to thank you for being present everyday at ACTA. Not (only) for the candies that where available whenever a break was needed; mainly for your strength! I deeply admire the things you achieve on an everyday

basis that have been an example for me to overcome difficult moments. Maurits, thanks a lot!

Dear professor Farina, Dear Dario, your Italian temperament and your hard work gave a significant initial boost during my stay at the Center for Sensory-Motor Interaction at Aalborg. Our different background was only an advantage that resulted in a lot of data and two of the studies included in this thesis. Moreover, your way of pioneering thinking on data organization and analysis has influenced all my future studies.

Dear professor Türker, Dear Kemal, you gave me the chance to work in a fine laboratory in Izmir, but also visit places with huge historical load for Greek people. My fears when moving to Izmir immediately disappeared when meeting you and your group in person. I will always remember the nice moments and the extremely fruitful collaboration at the "other side of the Aegean".

Dear professor Droukas, thank you for giving me the passion for the field of Orofacial Pain and your support and advice during my career!

Dear dr W. de Boer, Beste Wim, thank you very much for your knowledge and your advice. Your medical background was a source of very interesting discussions.

Beste Ko and Bart, your knowledge and your technical skills were extremely important for the studies performed at the laboratories of our department. I am sincerely grateful for all your work.

Beste Marylee and Wendy, thank you for your knowledge and experience that improved not only my patient care but also myself.

Beste Hanneke, Samara, Elaine, Inge, Rianne, and Els, you have always been the support of my work at OKI. Thank you very much for all your daily contribution on all the aspects of the work at OKI.

Lieve Ghizlane, our common roots around the Mediterranean Sea meant a lot of cultural similarities. This created a special connection between us two. We also shared our ambition for working on research, patient care, private practice, and at the same time trying to combine free time on our busy schedule. Thanks for the inspiring moments!

Lieve Annemiek, we really had something like parallel research lives inside ACTA. And you were always the positive energy and power of the PhD room. Thank you for giving me

the feeling that you were a discreet person I could always trust. And this always gave me a relaxed feeling.

Lieve Stani, we both left our countries to study and pursue our dreams in the Netherlands. This made us share our ambition and many difficult moments. Thanks for all the discussions and nice moments we had together.

Dear Carlijn van der Lugt, Dear Alwine te Veldhuis, Dear Emeline te Veldhuis, Dear Carolina Marpaung, Dear Maria Valiente-Lopez, Dear Adriaan Klitsie, Dear Magdalena Osiewicz, Dear Hasti Parvaneh, Dear Mohamed Magdy Attallah. Dear Georgine Vervoorn-Vis, Dear Erin Suijkerbuijk, Dear Adamantia Byraki, Dear Jessika Chin Jen Sem, you have been the young heart of OKI. Thanks for the inspiring moments inside and outside ACTA.

A special thanks to the Research Institute of ACTA and especially Vincent Everts, Martijn van Steenbergen, and Martine Meijer, thank you for your support throughout my PhD research.

Beste Daan and Uyen, together with the entire staff of the Referral practice for Endodontology and Gnathology Hoorn, I am grateful for the opportunity you gave me to increase my clinical experience in a very nice working environment!

All my Greek friends, and especially Kostas Syriopoulos, Chryssa Papagianni, Socratis Katsamakis, Maria Nikolopoulou, Kostas Kouroupis, Dimitris Papapanagiotou, Anna Louropoulou, Pavlos Papakonstantinou, Lida Kalogeraki, Victoria Ntrouka thank for your presence in Amsterdam that made the distance to Greece seem shorter.

A special thanks to everybody that participated and contributed to the studies included in this thesis. Without your contribution it would have been impossible to complete this work!

Dear Yiannis Vergoullis and Cathy Badell, thank you very much for giving me the opportunity to offer my knowledge and experience in relieving orofacial pain of patients at the Vergoullis Dental Clinic in Rodos. This has been a great chance for me to combine my work with a monthly visit to my family, something extremely important for my emotional balance.

Beste Peter, Miranda, Robert, and Maarten Wetselaar, you have always been my family in the Netherlands! Since my first visit to your house in 2004 we have spent several moments together: moments of hard work, of deep emotions, of worries, of joy. We celebrated successes, we discussed our difficult moments, we made plans for the future. All these

resulted in a strong friendship through the years. A small gesture of my sincere gratefulness is the dedication of this thesis to you all. Because words cannot fully express everything you offered me all these years…

Dear Leandros, Stefanos, Thanasis, and Theodore, your friendship and our common ambitions go back in several years now. The time we spent together gave me a lot of strength to go on with my work and complete this thesis!

A special thanks to Laertis Vassiliou for his help and contribution to the design and the illustration of the cover of this book!

Dear Roula Fotopoulou and Angeliki Tsiliagkou, you were always there to support Katerina, Roula, in combination with my professional choices. Knowing that, always gave me a safe feeling when being abroad. Thank you for all your support!

My beloved grandmother, Nina Kountouri, thank you very much for your support since my school years. You have always been the strong reference point for the entire family all these years.

My dear parents Thomas and Evangelia Koutris, I know that you had to make big sacrifices to support my studies in Greece and abroad. But you never complained… You were always present, understanding everything, even though I always tried to inform you only about the good news and hide the difficult moments. Thank you very much for the way you brought me up and all the principles you inherited me!

My beloved brother Thodoris and my beloved sister Katerina, I was always the "little brother" of the family spending more time studying and at home than going out. We were brought up together sharing difficult but also happy moments. Thank you for being present all these years.

Dear Thomais Zoannou, I hope that you are able to see/feel the completion of this work. You always encouraged and motivated me to study and work hard…

My beloved Katerina, without your support, none of the things I have accomplished during the last years would have been possible… I love you! My beloved little Roula, you were the reason for me to try harder and harder to finalize this thesis! A special dedication of this work to you both!