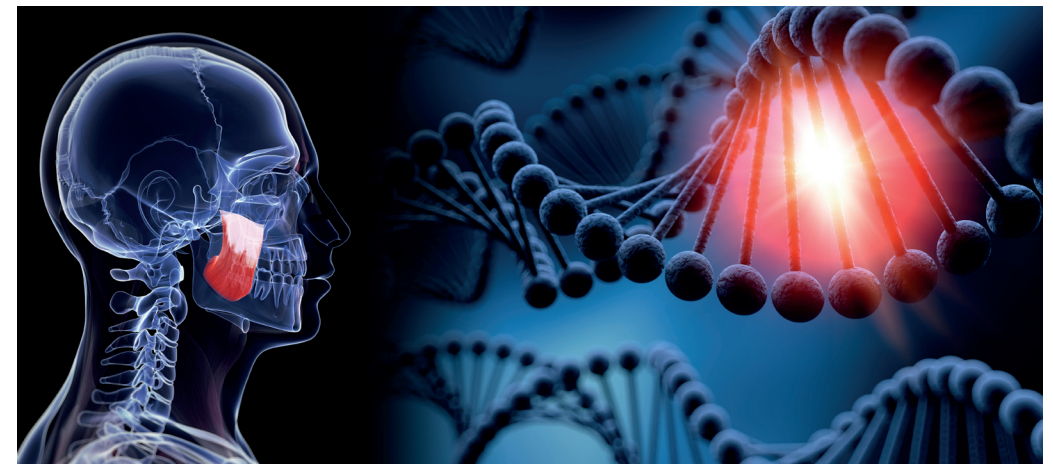


Thesis for doctoral degree (Ph.D.)
2017

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Biomarkers in chronic and experimental human muscle pain



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Sofia Louca Jounger

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**Karolinska
Institutet**



**Karolinska
Institutet**

Department of Dental Medicine
Karolinska Institutet, Stockholm, Sweden

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Stockholm 2017

Cover image: Illustration of masseter muscle pain from a microscopic and a macroscopic perspective, from genes to tissue damage.

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*To my beloved family, and most of all,
my husband, Pontus*

Biomarkers in chronic and experimental human muscle pain

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By

Sofia Louca Jounger

Principal Supervisor:

Professor Malin Ernberg
Karolinska Institutet
Department of Dental Medicine
Section of Orofacial Pain and Jaw Function

Opponent:

Associate professor Märta Segerdahl
Clinical Research and Development, Neurology
H. Lundbeck A/S
Valby, Denmark

Co-supervisor(s):

Associate professor Nikolaos Christidis
Karolinska Institutet
Department of Dental Medicine
Section of Orofacial Pain and Jaw Function

Examination Board:

Associate professor Albert Crenshaw
Gävle University
Department of Department of Occupational and
Public Health

Professor Thomas List
Malmö University
Faculty of Odontology
Department of Orofacial Pain and Jaw Function

Associate professor Gunilla Brodda Jansen
Karolinska Institutet
Department of Clinical Sciences, Danderyd
Hospital (KI/DS)

Professor Martin Schalling
Karolinska Institutet
Department of Molecular Medicine and Surgery
(MMK)

Professor Zsuzanna Wiesenfeld-Hallin
Karolinska Institutet
Department of Department of Physiology and
Pharmacology

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ABSTRACT

The main aim of this thesis was to improve the knowledge of the peripheral mechanisms that may participate in the underlying pathophysiology of chronic temporomandibular disorders (TMD) myalgia i.e. pain that is experienced locally in the jaw muscles and has myofascial trigger points. The project examined the effect of the 5-hydroxytryptamine type 3 (5-HT₃)-antagonist granisetron on experimentally induced muscle pain and whether specific genetic variants i.e. polymorphisms (SNPs) in the serotonergic system influences pain perception and the pain reducing effect of granisetron. The project also investigated the relationship between certain biomarkers; serotonin (5-HT), glutamate, metabolites and pro- and anti-inflammatory cytokines in jaw muscle pain.

One part examined the 5-HT₃- receptor antagonist granisetron, and its effect on experimentally induced masseter muscle pain in healthy participants. Also, whether certain polymorphisms in the serotonergic system are involved and may influence the pain response and the efficacy of granisetron. The SNPs (rs1062613, rs1176744) in the HTR3A/B genes were therefore investigated. 0.5 mL granisetron (Kytril® 1 mg/ml) or placebo (isotonic saline, 9 mg/mL) was injected in the masseter muscles in a randomized, placebo-controlled and double-blinded order, followed by a bilateral painful injection of either: a) acidic saline (0.5 mL, 9 mg/mL, pH 3.3) or b) hypertonic saline (HS, 0.2 mL, 58.5 mg/mL). The pain variables; pain intensity, pain duration, pain area and pain pressure threshold (PPT) were assessed.

Another part in this project, used a microdialysis technique in order to investigate the intramuscular levels of several biomarkers in the masseter muscle, at rest and after experimentally induced muscle pain. HS injections and static tooth-clenching were used as experimental pain models in healthy, pain-free participants and in patients with TMD myalgia. The biomarkers and metabolites analyzed were; 5-HT, glutamate, lactate, pyruvate, glucose, glycerol as well as the pro- and anti-inflammatory cytokines; IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7 IL-8, IL-10, IL-12, IL-13, TNF, IFN- γ and GM-CSF.

The results showed that granisetron had a pain reducing effect on experimentally induced masseter muscle pain, both by acidic saline and HS injections. The pain intensity, pain duration and pain area were significantly lower on the side pre-treated with granisetron, but did not have any effect on the PPT. Also, there were sex differences in pain variables and in response to granisetron. The 5-HT₃ polymorphisms did not influence any pain variables in general or the pain reducing effect of granisetron. However, there were sex differences in regards to pain variables and the efficacy of granisetron. Women had higher pain intensity and larger pain area after experimentally induced masseter muscle pain, and less pain

reduction (pain intensity, duration and area) of granisetron in specific genotypes of the 5-HT₃ polymorphisms.

Intramuscular microdialysis in the masseter muscles of healthy participants showed increased levels of 5-HT, glutamate and glycerol after evoked muscle pain with a HS injection, and 5-HT correlated positively to pain. In TMD myalgia patients, there were higher levels of the pro- and anti-inflammatory cytokines IL-6, IL-7, IL-8 and IL-13, throughout the microdialysis compared to healthy controls. The cytokines IL-6, IL-7, IL-8, IL-13 and TNF increased in response to experimental tooth-clenching in patients, and IL-6 and IL-8 increased in healthy controls. TMD myalgia patients reported higher pain and fatigue after tooth-clenching compared to controls. However, no correlation between the cytokine levels and pain and fatigue were found.

In conclusion, the results of this thesis showed that granisetron had a pain reducing effect on experimentally evoked masseter muscle pain, with a generally better effect in men. None of the 5-HT₃ polymorphisms investigated in this thesis, seemed to influence the experimentally induced muscle pain or the positive effect of granisetron. Nevertheless, there were some indications of gene-to-sex interactions in pain variables and granisetron effects. Therefore, one cannot completely exclude the possibility that polymorphisms in the serotonergic system may influence, predict or be a risk factor in developing chronic muscle pain. Further research is needed to systematically investigate multiple 5-HT polymorphisms in order to draw any further conclusions.

Further, the levels of the biomarkers 5-HT, glutamate and glycerol, increased after experimentally induced muscle pain in the masseter muscles, but without any sex differences. In addition, patients with TMD myalgia constantly had elevated levels of the pro- and anti-inflammatory cytokines IL-6, IL-7, IL-8 and IL-13 compared to healthy controls. The cytokine levels of IL-6, IL-7, IL-8, IL-13 and TNF increased in patients after tooth-clenching, and IL-6 and IL-8 increased in controls. This indicates that muscle inflammation could be involved in the multifactorial pathophysiology of chronic muscle pain. However, no correlations between the cytokine levels and pain and fatigue were found, indicating that there is no direct cause-relation effect between increased pain and cytokine release. Other peripheral mediators and mechanisms, such as central sensitization can therefore not be ruled out in the pathophysiology of chronic TMD myalgia.

Key words

5-HT, Biomarkers, Bruxism, Cytokines, Genetic, Granisetron, Hypertonic saline, Masseter muscle, Muscle pain, Myalgia, Pain, Pain threshold, Polymorphism, Temporomandibular disorders (TMD).

LIST OF PUBLICATIONS

1. **S. Louca**, M. Ernberg, N. Christidis.
Influence of intramuscular granisetron on experimentally induced muscle pain by acidic saline.
Journal of Oral Rehabilitation 2013 40; 403-412.
2. **S. Louca Junger**, N. Christidis, B. Hedenberg-Magnusson, T. List, P. Svensson, M. Schalling, M. Ernberg.
Influence of 5-HT₃ polymorphisms on experimental pain and the effect of granisetron.
PLoS ONE 2016, 11(12): e0168703.
3. **S. Louca**, N. Christidis, B. Ghafouri, B. Gerdle, P. Svensson, T. List, M. Ernberg
Serotonin, glutamate and glycerol are released by hypertonic saline injections in human masseter muscles – a microdialysis study.
The Journal of Headache and Pain 2014, 15:89.
4. **S. Louca Junger**, N. Christidis, P. Svensson, T. List, M. Ernberg
Increased levels of intramuscular cytokines in patients with jaw muscle pain.
The Journal of Headache and Pain 2017, 18:30.

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LIST OF ABBREVIATIONS

5-HT	5- hydroxytryptamine i.e. serotonin
ASIC	Acid-sensing ion channels
CNS	Central nervous system
DC/TMD	Diagnostic Criteria for temporomandibular disorders
DNIC	Diffuse noxious inhibitory control
H⁺	Protons
HS	Hypertonic saline
HTR3	5-hydroxytryptamine receptor 3
IBS	Irritable bowel syndrome
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IASP	International association of the study of pain
IFN-γ	Interferon gamma
IL	Interleukin
TMD	Temporomandibular disorders
MVCF	Maximal voluntary clenching force
NRS	Numeric rating scale
PCR	Polymerase chain reaction
PNS	Peripheral nervous system
PPT	Pressure pain threshold
PSS	Perceived stress scale
RDC/TMD	Research diagnostic criteria for temporomandibular disorders
RR	Relative recovery
SNP	Single nucleotide polymorphism

SP	Substance P
STAI	State-trait anxiety inventory
TMD	Temporomandibular disorders
TNF	Tumor necrosis factor
VAS	Visual analogue scale
WAD	Whiplash associated disorders

INTRODUCTION

What is pain? Throughout history, man has tried to define and describe pain. The ancient Greeks considered pain to be an emotional experience and was often viewed as an external punishment. Already in the 600 BC in Egypt, the historian and Bible writer Jeremiah, described pain as coming from deep within the intestines and associated pain with strong emotions. He asked: “why is my pain chronic and my wound incurable?”

Pain is a word that evokes various associations and emotions depending on experiences. It is always subjective and causes both physical and emotional harm, affected by psychological factors, with feelings of disappointment, unhappiness, guilt, anxiety and depression (Kelley and Clifford, 1997). The modern definition of pain is “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (International Association for the Study of Pain) (Merskey and Bogduk, 1994).

Nearly 20 % of the European population suffer from chronic pain (Breivik et al., 2006). The most common pain conditions in the orofacial area are temporomandibular disorders (TMD), with a prevalence of approximately 10-15 % in the adult population (Isong et al., 2008; LeResche, 1997) and are twice as common in women as in men (Dao and LeResche, 2000; Von Korff et al., 1988). TMD are chronic pain conditions localized to the jaw muscles and/ or temporomandibular joints and includes symptoms like, restricted mouth opening, pain upon chewing, muscle soreness, pain referral and headache (Lund et al., 2001; Sessle, 1999) leading to a decreased quality of life (Hallberg and Carlsson, 2000; Thomas, 2000). In addition to individual suffering, it is a big problem for the society, with increased work absence and health care costs (Von Korff et al., 1990; Yokoyama et al., 2007).

TMD are thought to be triggered by several risk factors such as psychosocial, autonomic and genetic factors (Fillingim et al., 2011). The most common subtype is myalgia with pain locally in the jaw muscles with myofascial trigger points (Gerwin, 2001). One risk factor suggested to contribute to TMD myalgia is tooth-clenching/ grinding (bruxism), leading to an overload with decreased blood flow and ischemia causing a release of algogenic substances and thereby cause pain (Mense, 1993, 2003). Serotonin (5-HT) as an example, is reported to be higher in patients with chronic myalgia than in healthy controls (Ernberg et al., 1999; Rosendal et al., 2004b). Also central mechanisms are thought to contribute to bruxism by disturbances in the dopaminergic system (Lobbezoo and Naeije, 2001). However, the knowledge is limited behind the peripheral and central mechanisms of chronic musculoskeletal pain.

The pathophysiological mechanisms that underlie chronic myalgia and why it is more prevalent in women is still not understood. Increased knowledge behind chronic muscle pain conditions will help to improve the diagnosis, and may lead to new therapies. This in turn may eventually lead to a reduced need of health care resources and health care costs and, not least, less suffering for the individual.

Classification of pain

Pain is a defense mechanism providing the body necessary information when potential or actual injury occurs (Dubin and Patapoutian, 2010). Pain can be classified by the duration i.e. acute or chronic pain (Treede et al., 2015). Since chronic pain consists of various pain conditions, a clarification of the concept and classification of chronic pain was recently published by IASP (Treede et al., 2015). A compilation of various chronic pain conditions was done including; chronic primary pain, chronic cancer pain, chronic postsurgical and posttraumatic pain, chronic neuropathic pain, chronic headache and orofacial pain, chronic visceral pain and chronic musculoskeletal pain (Treede et al., 2015). In this classification TMD pain categorizes under the subgroup of chronic headache and orofacial pain.

Acute pain

Acute pain is a type of pain that begins suddenly, has a short duration and a distinct and sharp character. It alerts and warns of disease or threat to the body. The normal healing time is within a few weeks (Treede et al., 2015). Acute pain can be caused by many various circumstances such as; surgery, broken bones, burns or cuts or labor and childbirth. If acute pain is not treated, it may lead to chronic pain.

Chronic pain

Chronic pain is an on-going pain that persists and lasts longer than 3 months, despite the fact that the underlying damage has healed. The etiology is unclear, but it might have originated with an initial trauma or injury leading to an activation of the acute warning system with pain signals that remains active in the nervous system. The severity of pain can be classified on pain intensity, pain-related distress and functional impairment. (Treede et al., 2015).

Chronic headache and orofacial pain

The definition of chronic headache and orofacial pain is headaches or orofacial pains that occur on at least 50 % of the days during at least 3 months (Headache Classification Committee of the International Headache, 2013; Treede et al., 2015). TMD are the most

common chronic orofacial pain conditions and have a nociceptive and neuropathic character (Benoliel et al., 2012; Schiffman et al., 2014). Other orofacial pain conditions included in this subcategory are; post-traumatic trigeminal neuropathic pain, persistent idiopathic pain and burning mouth syndrome. However, several of these pain conditions are cross-referenced to primary chronic pain and chronic neuropathic pain (Treede et al., 2015).

Pathways of orofacial pain

In the orofacial region, pain is mediated by inputs from the fifth cranial nerve (V) called the trigeminal nerve. It contains three branches that innervates the craniofacial tissues; The Ophthalmic (V1), the Maxillary (V2), and the Mandibular (V3) nerves. When a painful stimuli occurs, nociceptors i.e. sensory neurons (nerve cells) are activated, transforming the stimuli into electrical signals. The signals are passed on via the primary afferent neurons in the trigeminal nerve through the gasserian ganglion, to terminate in the subnucleus caudalis, which is considered to be the main site of dispatch information from the craniofacial region. In the subnucleus caudalis, the first order neurons synapse on the second order neuron and transmits the information through the neospinothalamic tract or paleospinothalamic tract to the thalamus. In the thalamus, it synapses on the third order neurons before it reaches the cerebral cortex, where pain is experienced (Sarnat and Laskin, 1992).

There are two different types of axons transmitting pain, A-delta and C-fibers (Dubin and Patapoutian, 2010). A-delta fibers are myelinated and transform the signals rapidly with a low threshold for pain leading to acute and sharp pain, and terminates in the ventral posteromedial nucleus. They respond to mechanical and thermal stimuli. C-fibers are unmyelinated and respond to chemical, mechanical and thermal stimuli and terminates in the intralaminar nuclei. They have a high threshold for pain leading to slow and burning pain. The stimulus (thermal, mechanical, or chemical) gets transduced into electrical impulses by proteins in the membrane of these nociceptors, which in turn are transmitted along the peripheral and central axon of the nociceptor into the central nervous system (CNS) where the signals gets interpreted (Dubin and Patapoutian, 2010).

Biomarkers

For many years, the definition of a biomarker has been discussed. According to one review of the literature regarding this matter, they have come to the conclusion that a biomarker is something that can be measured and evaluated and is involved in biological processes such as pain, inflammation, pharmacological response as well as therapeutic intervention (Ptolemy and Rifai, 2010).

When a tissue damage or invasion of pathogens occurs, an inflammatory process starts, which is a cascade of complex series of immune reactions. The inflammatory process is initiated to establish healing and repair. In the clinic, inflammation is characterized by the five classical cardinal symptoms; rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), and functio laesa (loss of function). The first reaction of an acute inflammation is the release of vasodilators and chemotactic factors like histamine, which leads to increased permeability and blood flow at the injured site. This is followed by migration of phagocytes and serum proteins through the cell walls which destroy bacteria. Several inflammatory biomarkers and mediators such as; cytokines, 5-HT, substance P (SP) and bradykinin activates the peripheral nociceptors either direct or indirect and cause pain (Dray, 1995).

Cytokines

Cytokines are small proteins involved in the immune system. Cytokine means “cell movement” and is a generic name including; lymphokines, monokines, chemokines, and interleukins (IL). Cytokines are produced in muscle cells, immune cells (T-cells, B-cells and macrophages) and other cells like fibroblasts and endothelial cells. There are both pro- and anti-inflammatory cytokines and they are often released in a cascade in response to tissue damage (Zhang and An, 2007). The pro-inflammatory cytokines for example stimulate its target cell to produce and release other cytokines initiating the inflammation, while anti-inflammatory cytokines control the pro-inflammatory cytokine response (Zhang and An, 2007).

The pro- and anti-inflammatory cytokines interact with each other in a balanced matter, in order to fight an infection and promote proper wound healing. A possible peripheral factor in chronic muscle pain conditions may be an imbalance between pro- and anti-inflammatory cytokines, promoting or maintaining pain (Figure 1). Previous studies have showed that pro-inflammatory cytokines seem to be involved in chronic pain conditions (Koch et al., 2007) while anti-inflammatory cytokines have an analgesic effect (Uceyler et al., 2006).

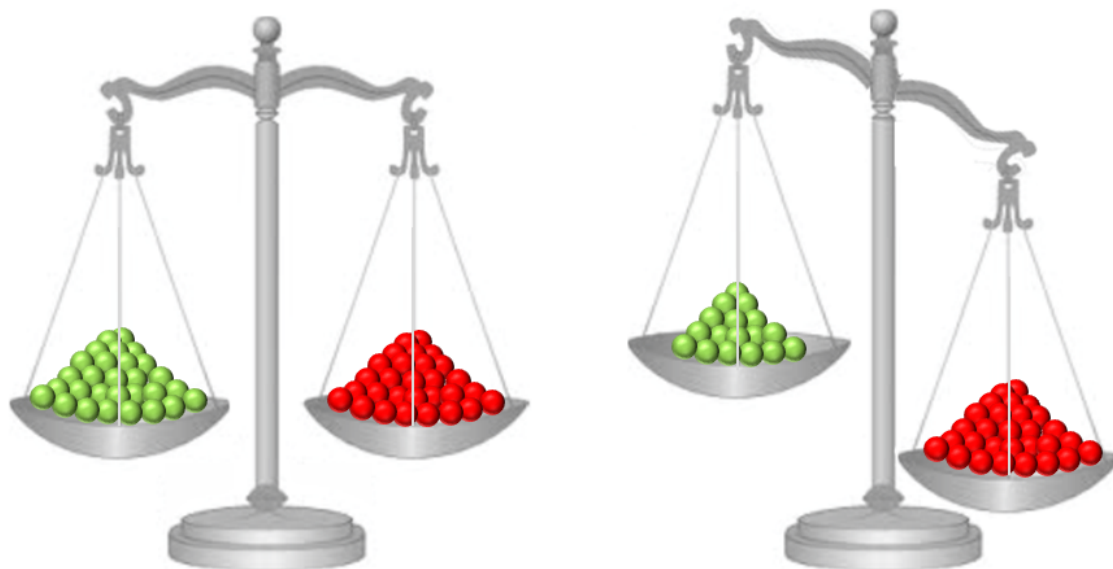


Figure 1. Illustration of the balance between pro- and anti-inflammatory cytokines. In a normal healthy condition, the pro- and anti-inflammatory cytokines interact with each other in a balanced matter promoting a stable environment (to the left). If an imbalance between pro- and anti-inflammatory cytokines occurs (to the right), an inflammatory response is triggered, which may play an important role in induction and maintenance of chronic pain.

The major anti-inflammatory cytokines are; IL-4, IL-6, IL-10, IL-11, and IL-13 (Opal and DePalo, 2000). IL-4 is produced by T cells, mast cells, B cells and stromal cells and can inhibit and down-regulate the effect of pro-inflammatory cytokines such as; tumor necrosis factor (TNF), IL-1 β , IL-6, IL-8 and nitric oxide production (Opal and DePalo, 2000). IL-6 acts both as a pro- and anti-inflammatory cytokine, involved in nociception, hyperalgesia and sickness response (McMahon et al., 2005; Sommer and Kress, 2004; Watkins and Maier, 2005). IL-6 can control the levels of pro-inflammatory cytokines (Xing et al., 1998) either by promoting or inhibiting the production. For example, IL-6 can inhibit the production of the cytokines Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon (IFN)- γ and also some key inflammatory responses by blocking the synthesis of TNF and IL-1 (Barton, 1997). Previous studies have shown that the levels of IL-6 are increased in patients with whiplash-associated trapezius myalgia (Gerdle et al., 2008b) and also in subjects with myofascial trigger points in the trapezius muscle compared to controls (Shah et al., 2008; Shah et al., 2005).

Further, IL-10 together with IL-4 are known for inhibiting the cytokines IL-2, TNF and IFN- γ (Opal and DePalo, 2000; Zhang and An, 2007). A previous study showed that patients with chronic widespread pain had low blood levels of the anti-inflammatory cytokines IL-4 and IL-10, suggesting that the lack of anti-inflammatory cytokines may play a role in the pathogenesis of chronic widespread pain conditions (Uceyler et al., 2006).

Another anti-inflammatory cytokine is IL-13, which is mainly produced by T cells and can inhibit the expression of nitric oxide and the pro-inflammatory cytokines IL-1, IL-6, IL-8, IL-10 and IL-12 (Lin et al., 2000). Together with IL-4, they share a common cellular receptor (IL-4 type 1 receptor) and also similar functions (Lin et al., 2000).

Pro-inflammatory cytokines are mainly produced by macrophages and are thought to be involved in the pathophysiology of chronic pain conditions since these cytokines contribute to an up-regulation of the immune system by initiating the inflammatory cascade (Zhang and An, 2007). A previous study showed that patients with chronic pain conditions such as; neuropathic, nociceptive and mixed pain, had higher levels of the pro-inflammatory cytokines IL-1 β , TNF, IL-2, IL-6 and IFN- γ in plasma, compared to healthy controls and that the increased levels correlated to pain intensity (Koch et al., 2007).

Further, in neuronal and glial cells, injections of IL-1 β in rats and rabbits increased the production of SP and prostaglandins (Jeanjean et al., 1995; Schweizer et al., 1988), which are well-known inflammatory mediators triggering nociceptors indirectly and therefore cause pain (Mense, 1993; Wall and Melzack, 1994). It has also been shown that IL-1 β directly sensitizes and activates nociceptors in primary afferent neurons (Binshtok et al., 2008). In muscle diseases such as inflammatory myopathies, IL-1 β together with TNF seem to be up-regulated (Kuru et al., 2000; Lundberg, 2000). TNF acts via the TNF receptors 1 and 2 (TNFR1 and TNFR2) (Zhang and An, 2007) and seems to play an important role in the inflammatory cascade and immune response by regulating the production of other pro-inflammatory cytokines, fibroblasts and up-regulation of receptors. For example, TNF can promote the production of IL-6 (Sommer and Kress, 2004). Moreover, intramuscular injections of TNF and IL-6 respectively in the gastrocnemius muscle of rats induced a long-lasting hyperalgesia (Dina et al., 2008; Schafers et al., 2003). Also, previous study showed an association with higher levels of TNF and depression in healthy men (Suarez et al., 2002).

Another pro-inflammatory cytokine involved in the inflammatory response is IL-7. It has been shown to be elevated in some inflammatory disorders such as; rheumatoid arthritis, but also stable and unstable angina (Damas et al., 2003; Harada et al., 1999). Further, IL-8 is a chemo attractant and activates neutrophils at the site of inflammation (Bickel, 1993). Previous studies report higher levels of IL-8 in plasma and cerebrospinal fluid of patients with TMD and widespread palpation tenderness and fibromyalgia compared to controls (Gur et al., 2002; Kadetoff et al., 2012; Kosek et al., 2015; Slade et al., 2011b). Furthermore, GM-CSF contributes to a normal inflammatory cytokine response by its recruitment of leukocytes (Lin et al., 2000). Also, it has been shown that GM-CSF delays the apoptosis of macrophages and neutrophils (Fanning et al., 1999). In a previous study, injections of GM-CSF caused inflammatory pain in mice (Cook et al., 2013).

With this in mind, cytokines are undoubtedly important in the progress and maintenance of neuropathic and inflammatory pain, thus their role in chronic myalgia are of great interest.

Glutamate and metabolic mediators

Glutamate is one of the body's twenty amino acids and is found in every part of the body and acts as a neurotransmitter by targeting its receptors; N-methyl-D-aspartate receptor (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA). When glutamate binds to its receptors in the peripheral nervous system (PNS), peripheral sensory afferents are activated and pain is induced (Ren and Dubner, 2010). Several studies have shown increased interstitial levels of glutamate in patients with TMD myalgia and trapezius myalgia compared to healthy controls, but also that glutamate correlated positively to pain (Castrillon et al., 2010; Rosendal et al., 2004b). Furthermore, intramuscular injections of glutamate evoked pain (Svensson et al., 2005). Also, painful injections in the biceps muscle with hypertonic saline (HS) caused a release of glutamate (Tegeder et al., 2002).

Further, the metabolic mediators lactate and pyruvate participate in the final steps of the glycolysis (Essen and Kaijser, 1978), hence they are important in the metabolic pathway. The concentration of lactate may increase rapidly during intense physical exercise, but the increase is rapidly passing (Essen and Kaijser, 1978). Previous microdialysis studies show altered levels of lactate and pyruvate in the painful trapezius muscle compared to healthy controls, indicating that they may also participate in chronic orofacial muscle pain (Gerdle et al., 2010; Ghafouri et al., 2010; Rosendal et al., 2004a). One suggestion for the altered levels are, decreased oxygenation locally in the muscle leading to either increased production or reduced degradation of the metabolites (Rosendal et al., 2004a).

Serotonin

5-HT is a monoamine neurotransmitter, important in pain mediation both peripherally and centrally (Zeitz et al., 2002). 5-HT derives from tryptophan (Giordano and Schultea, 2004) and is mainly found in the gastrointestinal tract where 90 % is located, but also in blood platelets and in the CNS. In the CNS it has various functions including regulation of mood, appetite, sleep and cognitive functions such as; memory and learning, but is also involved in pain inhibition (Lesurtel et al., 2008). 5-HT can stimulate the pain descending inhibition system by activating inhibitory interneurons that release endogenous opioids and gamma-aminobutyric acid (Giordano and Schultea, 2004; Sommer, 2006). In the PNS however, 5-HT acts as a pain inducer by activating some of its receptors or by promoting the release of SP and glutamate which activate and sensitize nociceptors leading to a sensation of pain (Mense, 1993, 2003).

There are seven 5-HT receptor classes (5-HT₁- 5-HT₇) with a number of receptors in each class, with a total of at least fifteen different receptors (Ernberg, 2009; Glennon and Dukat, 1991). 5-HT₃ is thought to be the most important receptor for pain modulation and with a therapeutic potential (Mackie et al., 2000; Ruano et al., 2007). It is the only ligand gated ion channel among the 5-HT receptors. The activation of the receptor facilitates the influx of sodium, potassium and calcium and is mapped to chromosome 11. 5-HT₃ have five subunits 5-HT_{3A-3E} each coded by their own gene (HTR3A-E).

Studies have shown that patients with myalgia in the masseter and trapezius muscles have higher levels of 5-HT than healthy controls (Dawson et al., 2015; Ernberg et al., 1999; Ghafouri et al., 2010) and that 5-HT correlated to pain (Ernberg et al., 1999; Gerdle et al., 2008b; Rosendal et al., 2004b). Also, intramuscular injections of 5-HT induced pain and hyperalgesia (Babenko et al., 1999; Ernberg et al., 2000b). Only two studies have investigated the interstitial release of 5-HT in experimental masseter myalgia, were repeated acidic saline injections and static tooth-clenching were used as experimental pain models. In both studies, no increased levels of 5-HT was reported (Dawson et al., 2015; Ernberg et al., 2013). However, TMD myalgia patients had constantly higher levels of 5-HT than healthy controls (Dawson et al., 2015).

To our knowledge, no previous study have investigate if jaw muscle pain induced by HS leads to a release of muscle biomarkers such as 5-HT, glutamate and other metabolic mediators. Since HS is a valid and commonly used experimental pain model, the same biomarkers, as in clinical pain, should elevate tentatively after a HS injection.

Granisetron is a 5-HT₃ antagonist and is a frequently used drug in radiotherapy- and chemotherapy-induced nausea and vomiting in patients with cancer, but also in IBS (Ahn and Ehrenpreis, 2002; Vrabel, 2007). There are several 5-HT₃ antagonist such as; ondasetron, dolasetron and tropisetron which all seem to have an equal effect and few side-effects. Granisetron however, has a higher affinity to the 5-HT₃-receptor and does not rely on the enzyme cytochrome P450 2D6 (CYP2D6) in the metabolism. Previous studies have shown that these 5-HT₃ antagonists have an effect on localized muscle pain in the lower back, neck and face (Christidis et al., 2008; Christidis et al., 2015b; Ettlin, 2004; Stratz and Muller, 2004). Thus, granisetron may be a useful therapeutic treatments for chronic myalgia.

5-HT₃ polymorphisms

In recent years, genetic factors underlying various diseases have received considerable attention. There are at least 358 genes thought to be relevant in pain and hyperalgesia (Smith et al., 2011). Although no single gene has been shown to cause chronic pain conditions, multiple genetic changes (polymorphisms) are thought to be involved. These

multiple genetic changes may occur when the genome is copied due to variations in a single nucleotide. A single base pair may get left out, added or substituted. These single base pairs substitutions create single nucleotide polymorphisms (SNPs). The variations in the DNA sequence can affect how we develop diseases, respond to drugs, vaccines and other agents (Alwi, 2005).

Several studies have related polymorphisms in the HTR3A/B genes to psychiatric disorders (Niesler et al., 2008), only a few studies have shown an association to chronic pain. For example, the HTR3A polymorphism was associated with an antidepressant response in paroxetine-treated patients (Kato et al., 2006) and showed an involvement in the etiology of eating disorders (Hammer et al., 2009). Further, a polymorphism in the HTR3B gene (rs1176744) was correlated to major depression in Japanese women (Yamada et al., 2006) and bipolar disorder (Hammer et al., 2012). The same SNP showed greater symptoms and anxiety in patients carrying the C/C genotype compared to patients with the T-allele (Kilpatrick et al., 2011). Also, in the HTR3B gene (rs1176744), subjects carrying the C allele showed an association to higher scores on the Pain Catastrophizing Scale, suggesting a role of 5-HT pathways in pain catastrophizing (Horjales-Araujo et al., 2013). In addition, it has been shown that polymorphisms of HTR3A/B serve as predictors for the effectiveness of 5-HT₃ antagonists (Niesler et al., 2008) and to the antidepressant response of the serotonin selective reuptake inhibitor (SSRI) paroxetine (Kato et al., 2006). Furthermore, subjects with a SNP (rs1176744) in the HTR3B gene, showed an increase response to 5-HT (Krzywkowski et al., 2008) indicating that subjects carrying this SNP could be more sensitive to pain.

Taken together, these findings indicate that polymorphisms in the HTR3A/B genes may be involved in the pathogenesis of depressive disorders and the effect of 5-HT₃ antagonists. Since pain and depression share the same pathways (Delgado, 2004), there are reasons to believe that the 5-HT polymorphisms also may be involved in the pathophysiology of chronic myalgia.

Experimental pain models

Experimental pain models are developed to mimic the clinical setting, in order to improve the knowledge of the pathophysiology underlying chronic pain conditions (Arendt-Nielsen et al., 2007). Human experimental pain models are the link between basic science and clinical research (Reddy et al., 2012). There are a number of different experimental pain models that can be divided into endogenous and exogenous methods. Endogenous methods means pain induced by natural, endogenous stimuli, and exogenous methods involve experimental pain induced by externally applied stimuli (Graven-Nielsen, 2006; Graven-Nielsen et al., 2003; Staahl and Drewes, 2004). The experimental pain models can be used in patients with pain conditions but also in healthy controls. An experimental pain model is

thought to trigger the nociceptive system by activating the peripheral nerve cells (Reddy et al., 2012). The pain evoked is then assessed and evaluated with different tools such as; the visual analogue scale (VAS), the numeric rating scale (NRS), pain drawings and pressure pain thresholds (PPT).

In this project we have used both endogenous and exogenous methods to study muscle pain, namely; acidic saline injections, HS injections (exogenous methods) and static tooth-clenching (endogenous method).

Acidic saline injections

Acidic saline is a solution with a lowered pH (pH 3.3- pH 5.2) by the supply of protons (H^+). H^+ play a key role in the upcoming experimental muscle pain by lowering the pH of the tissue, triggering the chemo sensitive nociceptors such as acid-sensing ion channels (ASIC1 and/or ASIC3) and/or the transient receptor potential vanilloid 1 on primary afferent neurons (Frey Law et al., 2008).

In animal studies, injections of acidic saline (pH 4) intramuscularly have been shown to be a successful experimental pain model causing a long-lasting hyperalgesia, mimicking chronic myalgia. In one study, repeated intramuscular injections of acidic saline into the gastrocnemius muscle of rats evoked a long lasting mechanical hyperalgesia with a duration up to 30 days (Sluka et al., 2001). In another study repeated injections of acidic saline (pH 4, 20 μ L) with 2 days apart, into the rat masseter muscle caused long-lasting mechanical allodynia for up to 38 days (Lund et al., 2010). However, in contrast, another study did not show any long-lasting allodynia in the rat masseter muscle after two repeated injections of acidic saline (pH 4, 150 μ L) 2–5 days apart (Ambalavanar et al., 2007). In a human study, mild to moderate muscle pain with pain referral and mechanical allodynia was induced by a single injection of a buffered acidic saline infusion (pH 5.2) into the tibialis muscle, that lasted for 20 min (Frey Law et al., 2008). Furthermore, at the same time our research group performed two experimental studies with acidic saline injections. In one of them, repeated injections in the masseter muscle failed to cause a long-lasting hyperalgesia in humans (Castrillon et al., 2013). In the other, which was a microdialysis study, injections of acidic saline in the masseter muscle did not cause the release of algescic substances such as 5-HT, glutamate, pyruvate, lactate and glucose (Ernberg et al., 2013).

Hypertonic saline injections

An often used experimental pain model is HS injections. It is a solution that contains 1 to 23.4 % NaCl compared to normal (isotonic) saline solution containing 0.9 % NaCl (in the human body). It is considered to be a valid model of TMD myalgia (Svensson et al., 2001b). It has an acute character and causes a pronounced sensation of deep, diffuse pain,

and pain referral (Graven-Nielsen et al., 2001; Jensen and Norup, 1992; Stohler and Lund, 1994; Vecchiet et al., 1993). The pain intensity and character depends on factors or variables such as the volume of HS solution, concentration and infusion rate (Graven-Nielsen, 2006; Graven-Nielsen et al., 1996). However, the painful sensation also depends on the individual differences for pain perception in the test group. The pain inducing effect of HS injections has been suggested to occur direct or indirect, by activation of sodium channels (Cairns et al., 2003), but also by the release of inflammatory substances such as; glutamate and SP (Garland et al., 1995; Tegeder et al., 2002).

Several previous studies have showed that biomarkers such as; 5-HT, glutamate and other metabolites (lactate and pyruvate), are higher in patients with chronic muscle pain conditions in the masseter and trapezius muscles compared to healthy controls (Castrillon et al., 2010; Ernberg et al., 1999; Gerdle et al., 2010; Ghafouri et al., 2010; Rosendal et al., 2004b; Tegeder et al., 2002; van Hall et al., 2002). However, in order to increase the validity of the experimental pain model, the same biomarkers released in clinical pain should also be increased after a HS injection.

Experimental tooth-clenching

Previous studies have suggested that self- reported tooth-clenching is a risk factor in developing TMD myalgia (Huang et al., 2002; Velly et al., 2003) by causing a disturbed blood flow due to overloaded muscles, leading to ischemia and the release of inflammatory biomarkers which activate peripheral afferents (Barr and Barbe, 2002; Monteiro and Kopp, 1989; Stauber, 2004). It is also suggested that repetitive muscle work may maintain chronic muscle pain due to temporal summation (Bennett, 2012). Previous studies have shown that excessive chewing evoked muscle pain and fatigue, suggesting that TMD pain is more alike an exercise-induced muscle pain (Koutris et al., 2009; Staahl and Drewes, 2004).

In another study, a 20-minute repetitive tooth-clenching task with 50 % of the maximum voluntary clenching force (MVCF), caused increased pain in TMD myalgia patients compared to healthy controls. Also, TMD myalgia patients had higher levels of 5-HT compared to healthy controls during the entire microdialysis, but it did not increase due to tooth-clenching, suggesting that other biomarkers may trigger the nociceptors and cause pain (Dawson et al., 2015).

AIMS

The general aim of this thesis was to increase our knowledge of the peripheral pain mechanisms and pathophysiology underlying TMD myalgia.

Specific aims

- To evaluate the effect of the 5-HT₃-antagonist granisetron on experimentally induced muscle pain.
- To investigate if 5-HT₃ polymorphisms contribute to pain perception and the efficacy of the 5-HT₃ antagonist granisetron on experimentally induced muscle pain.
- To investigate the role of muscle biomarkers (5-HT, glutamate, lactate, pyruvate, glucose and glycerol) and pro- and anti-inflammatory cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7 IL-8, IL-10, IL-12, IL-13, TNF, IFN- γ and GM-CSF) in the pathophysiology underlying TMD myalgia in a human experimental and a clinical study.

HYPOTHESES

The following hypotheses were tested:

Study I

- Muscle pain induced by two repeated acidic saline injections into the masseter muscle of healthy volunteers can be blocked by the 5-HT₃ antagonist granisetron.
- The pain-reducing effect by granisetron is better in men than in women.

Study II

- Polymorphisms in the serotonergic system are of importance for pain transmission and for the efficacy of the 5-HT₃ antagonist granisetron on experimentally induced muscle pain.
- There are sex differences due to specific HTR3A/B genotypes in pain response after experimentally evoked muscle pain and the analgesic effect of granisetron.

Study III

- Muscle pain induced by HS in the masseter muscle causes a significant release of 5-HT, glutamate, lactate, pyruvate, glucose and glycerol.
- The release of muscle biomarkers are higher in women than in men.

Study IV

- The levels of pro- and anti-inflammatory cytokines are significantly higher in patients with TMD myalgia both at rest and after a repetitive tooth-clenching task.
- The release of cytokines are correlated with pain intensity and fatigue.

MATERIALS AND METHODS

The methods and selection of participants were approved by the Regional Ethical review board in Stockholm, Sweden (*Study I*: 2008/362-31; *Study II*: 2011/1955-31/2; *Study III*: 2008/362-31; *Study IV*: 2009/2047-32;), the Medical Products Agency in Uppsala, Sweden (*Study I*: 2008-000746-32; *Study II*: 2011-006206-27, Dnr 151:2011/96710), the Swedish Data Inspection Board in Stockholm, Sweden (*Study II*: Dnr 54-2013) and the Local Radiology Committee (*Study III*: Dnr 11/08) at Karolinska University Hospital in Huddinge, Sweden.

All studies were conducted at the Department of Dental Medicine at the Karolinska Institutet, Huddinge, Sweden and followed the guidelines of the Declaration of Helsinki as well as the Good Clinical Practice guidelines. All participants were over 18 years of age and were recruited among staff, colleagues and students at the Department, but also by advertisements. All participants received written and verbal information of the study before participating and gave their written consent.

Healthy participants

A number of 134 healthy participants, 77 women and 57 men, participated in the studies (*I-VI*) (Table 1). The participants were age- and sex-matched.

Inclusion criteria were: age over 18 years and a good general health.

Exclusion criteria for all studies were: no current or history of pain from the orofacial region. In addition the exclusion criteria for *study I* were: a) migraine and/or tension-type headache; b) use of any kind of medication except for contraceptives 24 hours preceding the study day; c) smoking; d) pregnancy or lactation; and e) a history of allergic reactions to granisetron. For *studies II-IV* the exclusion criteria were: diagnosed systemic muscular or joint diseases such as: a) fibromyalgia; b) rheumatoid arthritis; c) whiplash-associated disorder; d) neuropathic pain or neurological disorders; e) pregnancy or lactation; f) high blood pressure and g) use of antidepressants or analgesics during the last three days.

Patients

Twenty female patients with TMD myalgia participated in *Study IV* (Table 1). The patients were referred to the Section of Orofacial Pain and Jaw Function at the Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden.

The inclusion criteria were; age over 18 years and a diagnosis of TMD myalgia according to the DC/TMD (Schiffman et al., 2014).

The exclusion criteria were: systemic muscular or joint diseases, such as a) fibromyalgia; b) rheumatoid arthritis; c) whiplash-associated disorder; d) neuropathic pain or neurological disorders; e) pain of dental origin; and f) use of analgesics of non-steroidal anti-inflammatory drugs during 48 hours before microdialysis.

Table 1. The number of healthy participants and their age (years). Values are expressed as number of participants and as mean (\pm SD) for age.

Study	I	II	III	IV
Healthy participants				
All	28	60	26	20
Men	14	30	13	0
Women	14	30	13	20
Age				
All	25.8 \pm 2.4	26.2 \pm 3.9	25.7 \pm 4.3	29 \pm 11
Men	27.8 \pm 6.2	27.1 \pm 4.4	25.7 \pm 4.3	0 \pm 0
Women	23.1 \pm 1.8	25.6 \pm 3.6	26.1 \pm 4.4	29 \pm 11
Patients (TMD myalgia)				
All	-	-	-	20
Men	-	-	-	0
Women	-	-	-	20
Age				
All	-	-	-	31.2 \pm 9.8
Men	-	-	-	0 \pm 0
Women	-	-	-	31.2 \pm 9.8

Methods

Assessment of pain

Visual analogue scale and Numeric rating scale

In the *studies I* and *II*, a 0-100-mm VAS scale was used to assess the pain intensity after experimentally induced muscle pain in the masseter muscles, and in *studies III* and *IV* a 0-10 NRS scale was used. The scales were marked with the end-points “no pain” and “the worst pain ever experienced”.

Pain drawings

Pain drawings were used to assess the pain area and or pain referral in *studies I-III*. Participants were instructed to mark their pain distribution on face charts with the lateral side of the head. For the analysis, the face charts were placed over a transparency with 1.5-1.5 mm squares and the full squares were counted. Squares partly inside the border i.e. two half squares or three 1/3 squares were added up to full squares, in line with previous study (Christidis et al., 2008). The area were expressed in arbitrary units (au).

Pressure pain threshold

PPT was assessed in all studies (*I-IV*) with an electronic pressure algometer (Somedic Sales AB, Höör, Sweden). The algometer was calibrated at study start and the zero level was balanced before each measurement. The tip of the algometer was 1 cm² and covered with a 1 mm thick rubber pad. A standardized pressure of 50 kPa/s was used. The tip was placed on the most prominent part of the masseter muscle and the participants were instructed to press a signal button as soon as the pressure turned into pain. The procedure was first tested on the right thumb on the dorsal side. The PPT was recorded bilaterally over the masseter muscles as well as over a reference point, which was the tip of the right index finger. The reference point was used in order to register if any possible systemic effects occurred by the treatments in *studies I and II*.

DNA analysis

In *study II*, a 4 ml blood sample was taken from a peripheral vein using a Vacutainer tube containing ethylenediaminetetraacetic acid solution. The blood sample was immediately stored at -80°C until analysis. If a blood sample was not able to be collected, a saliva sample was instead collected, using the OG-500 kits (DNA Genotek Inc, Ontario, Canada). The saliva samples were stored in room temperature according to the manufacturer's instructions. Prior to the analysis, DNA was extracted from blood or saliva using standard methods at the Department of Molecular Medicine and Surgery (MMK), Karolinska University Hospital Solna, Stockholm, Sweden. The HTR3A/B SNPs (rs1062613 and rs1176744) were genotyped with the Applied Biosystems Quantstudio 7 Flex Real-Time PCR System from Thermo Fischer Scientific, Carlsbad, CA by using allele specific Taqman minor groove binder probes labeled with fluorescent dyes Fluorescein and VIC, according to the manufacturer's protocol. Polymerase chain reaction (PCR) is a technique used to amplify a single copy or a few copies of a segment of DNA, creating thousands to millions of copies of a particular DNA sequence (Holland et al., 1991). This was done according to previous studies (Johansson et al., 2012; Nikamo et al., 2014; Nikamo et al., 2015).

Questionnaires

Questionnaires of psychological nature were assessed in *studies III and IV* in order to measure the level of anxiety and stress in adults. The questionnaires used were; the State-Trait Anxiety Inventory (STAI) and the Perceived stress scale-14 (PSS-14).

State-trait anxiety inventory

The Swedish version of the State-trait anxiety inventory (STAI) was used to assess trait-anxiety. The STAI questionnaire contains two scales with twenty questions to determine the anxiety level. The first scale measures anxiety of an event, and the second scale measures anxiety as a personal characteristic. The scores range from 20 to 80 where higher scores indicate higher levels of anxiety. Scores ≤ 30 indicate no or low signs of anxiety, but scores > 30 show high signs of anxiety. In both studies (*III, IV*) the focus was on a general and long-standing quality of trait anxiety since it is very common in patients with chronic pain (Spielberger, 1975). The Swedish version of the STAI was used (Forsberg and Bjorvell, 1993).

Perceived stress scale-14

The PSS-14 questionnaire comprises 14 stress-related questions of a general nature. It contains questions about feelings and thoughts during the last month, situations in life perceived as stressful and the current levels of stress. The maximum scoring is 56 where scores of 24 or below are considered as normal in healthy participants (Cohen et al., 1983). The Swedish version of the PSS-14 was used (Nordin and Nordin, 2013).

Microdialysis

Microdialysis is a minimally invasive technique used to study the release of biomarkers in different tissues in the body (Ungerstedt, 1991). The technique is similar to a capillary blood vessel where the molecules diffuse through endothelial cells to blood cells. The technique contains a microdialysis catheter connected to a micro infusion pump to perfuse the tissue with perfusion medium. On the tip of the catheter, a semipermeable membrane is glued, allowing molecules in the extracellular fluid to diffuse across the membrane and be collected by the catheter (Figure 2) (Lindfors et al., 1987). The cut off value of the probe, determines which molecules will pass through the membrane. The fluid (dialysate) collected by the catheter in microvials, is analyzed. This technique was used in *studies III and IV* to sample several biomarkers from the masseter muscle.

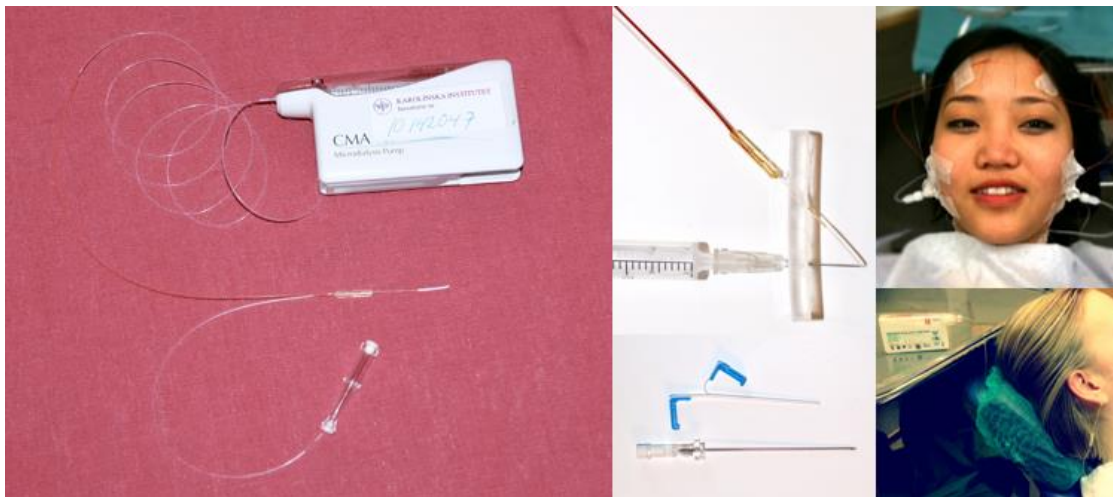


Figure 2. Illustration of the microdialysis technique containing a catheter with a semipermeable membrane at the tip, a micro infusion pump and microvials collecting the buffered ringer-acetate solution. In study III, the needle was inserted through a plastic guiding tool with pre-made holes (45° and 90° angles), confirming that the HS injection targeted the same location in the masseter muscle. In study IV, a split able introducer was used to insert the catheter intramuscularly.

Study III

Intramuscular microdialysis in the masseter muscle was done to sample 5-HT, glutamate, lactate, pyruvate, glucose and glycerol, and to estimate nutritive muscle blood flow. The most prominent point of the masseter muscle was palpated and chosen and a local anesthetic patch (EMLA® patch, lidocaine/prilocaine 25 mg/25mg, AstraZeneca AB, Södertälje, Sweden) was applied for 30 minutes. After removing the patch from the muscle and cleaning it with injection swabs (70 % isopropyl alcohol), a sterile flexible microdialysis catheter (Ø 0.5 mm; membrane length 10 mm, total length 30 mm; molecular cut off: 6 kDa MAB 11, Microbiotech AB, Stockholm, Sweden) was inserted in the muscle via a 6-mm thick sterile plastic plate (10 × 40 mm) with pre-made holes, one at 90° angle to the surface, and the other at 45° angle. The plastic plate was used so that the catheter was placed in close proximity to the needle in the muscle, in order to reassure that the HS injection targeted the same area in the masseter muscle (Figure 2). The catheter was inserted through the 45° angle canal and connected to a micro infusion pump (MAB 140, Microbiotech AB, Stockholm, Sweden) and perfusated with a buffered ringer-acetate solution containing 0.5 mM ringer-lactate. The perfusion rate was 5 µl/min. Dialysates of 120 µL were collected every 20 minutes in microvials. Samples were immediately frozen at -70°C. Relative recovery of the dialysates was determined by adding 3.0 µL [¹⁴C]-lactate (specific activity: 7.4 MBq/mL; PerkinElmer Life Sciences, Boston, MA, USA) (Scheller and Kolb, 1991), and the blood flow was estimated by adding 3.0 µL ³H₂O (Gerdle et al., 2008b; Rosendal et al., 2004b).

Study IV

Intramuscular microdialysis was performed in the masseter muscle to sample the pro- and anti-inflammatory cytokines; IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7 IL-8, IL-10, IL-12, IL-13, TNF, IFN- γ and GM-CSF. The most prominent point of the masseter muscle was chosen and anaesthetized superficially with a local injection (0.5 ml) of Xylocaine (20 mg/ml) making sure not to anaesthetize the underlying muscle. A sterile split able introducer was inserted at a 45° angle in the masseter muscle at a depth of approximately 40 mm from the skin surface. A sterile microdialysis catheter (High cut off Brain Microdialysis Catheter; membrane length 20 mm, total length 60 mm; molecular cut off: 100 000 Dalton, CMA 71 Microdialysis AB, Solna Sweden) was then inserted in the muscle, and the introducer was removed by splitting the plastic tube (Figure 2). A micro infusion pump (CMA 107, Microdialysis AB, Solna Sweden) was connected to the probe. The perfusion rate was 5 μ l/min with a ringer-acetate solution containing 0.5 mM ringer-lactate. Micro dialysates were sampled every 20 minutes in microvials. Samples were stored at -80°C.

Relative recovery

The dialysate samples do not reflect the true values of the extracellular concentration due to several factors; the flow rate, the diffusion rate through the tissue, the area and weight cut off of the dialysis membrane and the composition of the perfusate (Gerdle et al., 2014). Relative recovery (RR) describes the concentration of the dialysate in relation to the extracellular fluid. Factors that affects the RR are; a) the osmotic pressure b) the temperature and c) the concentration gradient (Dahlin et al., 2010). When small molecules such as 5-HT and glutamate are sampled, a cut off of 20 kilo Dalton (kDa) or less can be used, whereas for larger molecules such as cytokines, a higher cut off is required, usually 100 kDa. The interstitial concentration can be calculated by using the mathematic formula: $C_i = [C_d - C_p] / RR + C_p$ where C_d was the dialysate concentration and C_p , the perfusate concentration (Scheller and Kolb, 1991).

To determine the RR of each substance, 5 μ L of each dialysate or perfusate was pipetted into a counting vial with 3 μ L of scintillation fluid (High-flash Point, Universal LSC-Cocktail, ULTIMA GOLD™, PerkinElmer, Inc.) and vortexed. A formula was used to calculate the RR i.e. $(cpm_p - cpm_d) / cpm_p$, where cpm_p was counts per min of the perfusate, and cpm_d was counts per min of the dialysate. To calculate counts per minutes (cpm) a liquid scintillation beta counter (Beckman LS 6000TA; Beckman Instruments, Inc., Fullerton, CA, USA) was used for [14 C]-lactate and 3 H $_2$ O. Nutritive blood flow was estimated using 3 H $_2$ O with the formula: $1 / (cpm_d / cpm_p)$, where cpm_d was 3 H $_2$ O counts per min in the dialysate and cpm_p , in the perfusate (Gerdle et al., 2008b; Rosendal et al., 2004b). In *study III*, the RR and blood flow were analyzed for the whole microdialysis, for all time-points.

Analyzes of biomarkers

In *study III*, the concentrations of 5-HT were analyzed with high-pressure liquid chromatography, with electrochemical detection according to a previous study (Ghafouri et al., 2010). The limit of detection (LOD) for the 5-HT was 20 fmol/10 μ L. Other biomarkers (glutamate, glucose, lactate, pyruvate and glycerol) were analyzed with the ISCUS® analyzer (ISCUS, Dipylon Medical AB, Solna, Sweden). The limit of detection (LOD) of glutamate was 1.0 μ mol/L, for glucose and lactate 0.1 mmol/L, for pyruvate 10 μ mol/L and for glycerol 0.22 mg/mL. Concentrations that were 50 % below LOD were reported as having the same concentration as the LOD. Concentrations that were 50 % above LOD were reported as obtained. These analyzes were done at the Painomics Laboratory, Rehabilitation Medicine, Department of Medical and Health Sciences, Linköping University, Sweden.

In *study IV*, the concentrations of the cytokines were analyzed with Luminex technology and Bioplex® (Multiplex System, Bio- Rad) and multiplex immunoassay panels (Milliplex® map kit, Human High sensitivity, T cell magnetic bead panel, 96-well plate assay, Merck Millipore Darmstadt, Germany) according to the manufacturer's manual. The analysis were done at the research lab at the Department of Dental Medicine, Huddinge, Sweden. The LOD for each cytokine was; TNF, 0.43 pg/mL; IL-1 β , IL-2, IL-5 and IL-12, 0.49 pg/mL; IL-4, 1.83 pg/mL; IL-6, 0.18 pg/mL; IL-7, 0.37 pg/mL; IL-8, 0.31 pg/mL; IL-10, 1.46 pg/mL; IL-13, 0.24 pg/mL; IFN- γ , 0.61 pg/mL and GM-CSF, 1.22 pg/mL.

Experimental protocol

A randomized, placebo-controlled and double-blinded design was used for *studies I-III*, and a case control design for *study IV*. A randomization list performed by a computer was used to balance the injections and administration of granisetron or placebo in *studies I and II*, and the injections with HS or placebo in *study III*. The participants were seated in a relaxed position in a conventional dental chair during all experiments.

Study I

First, participants were screened with a clinical examination according to the Research Diagnostic Criteria for TMD (RDC/TMD) Axis I (Dworkin and LeResche, 1992) in order to establish that the inclusion criteria were fulfilled. Baseline recordings of PPT were assessed, and thereafter participants received a bilateral injection of acidic saline (9 mg/mL, pH 3.3) into the masseter muscles (day 1). An infusion pump (infusion rate 1200 μ L/min; Harvard Infusion Pump 22, Harvard Apparatus, Great Britain) was used in order to induce simultaneous bilateral pain. Two days later (day 3), pain intensity (VAS) and PPT were assessed in the masseter muscles. The masseter muscle on one side was then pre-treated with granisetron (Kytril®, 1 mg/mL, Roche, Stockholm, Sweden) and the contra-lateral side with placebo (isotonic saline, pH 6). Two minutes later, a bilateral simultaneous

injection of acidic saline followed. The needles were kept in the masseter muscles between injections to reassure that the treatment injections targeted the same site as the acidic saline injections. The pain evoked was assessed on a visual analogue scale (VAS) immediately after the injections and then every 15th second until pain had subsided to a maximum extent of 300 sec. Pain duration (sec) and pain area (au) were also assessed. The PPT recordings were made at baseline and 5, 15, 30, 45 and 60 minutes after the second injection of acidic saline. After 7 days (day 10), the pain distribution (au) and PPT were re-assessed.

Study II

Participants were first screened with a clinical examination according to the Research Diagnostic Criteria for TMD (RDC/TMD) (Dworkin and LeResche, 1992). Blood or saliva were sampled for genetic analyzes of the HTR3A/B polymorphisms (rs1062613, rs1176744). A bilateral injection of hypertonic saline (HS, 5.5 %, 0.2mL) was injected into the masseter muscles (injection 1) to evoke pain, using an infusion pump (infusion rate 1200 μ L/min; Harvard Infusion Pump 22, Harvard Apparatus, Great Britain). Thirty minutes later, the masseter muscle on one side was pre-treated with 0.5 mL of granisetron (Kytril®, 1 mg/mL, Roche, Stockholm, Sweden), and on the contralateral side with 0.5 mL of isotonic saline (9 mg/mL). Two minutes later, another bilateral HS injection (injection 2) was injected. Pain intensity (VAS) was assessed immediately after the injections and then every 15th second until pain had subsided to a maximum extent of 300 sec. After each HS injection, pain intensity, pain duration, pain area and PPT were assessed.

Study III

Participants were examined with a clinical examination according to the Research Diagnostic Criteria for TMD (RDC/TMD) Axis I (Dworkin and LeResche, 1992). After inclusion, questionnaires of psychological nature (STAI and PSS-14) were used to measure the level of anxiety and stress. Thereafter, bilateral intramuscular microdialysis in the masseter muscles was performed to sample 5-HT, glutamate, lactate, pyruvate, glucose and glycerol. Microdialysis was performed during 3 hours. After 2 hours of stabilization (trauma phase), HS (5.5 %, 0.2 mL) was injected into the masseter muscle on one side, and isotonic saline (placebo: 0.9 %, 0.2 mL) into the contra-lateral side, in close vicinity to the microdialysis catheter in a randomized order. Pain intensity (VAS), pain duration (sec) and pain area (au) were assessed. Furthermore, PPT was assessed, before and after microdialysis.

Study IV

First, a clinical examination according to the Diagnostic Criteria for TMD (DC/TMD) (Schiffman et al., 2014) was performed, then the maximal voluntary clenching force (MVCF) and PPT were assessed. After baseline registrations and local anesthesia, the microdialysis started. After 120 minutes of rest (trauma phase), baseline assessments of pain intensity and fatigue were made (120-140 min) followed by a 20-minute repetitive tooth-clenching task (140-160 min) at 50 % of MVCF (kg). A bite-force transducer (Aalborg University, Denmark) was used to assess the MVCF, and placed between the molars on the most suitable side, from a dental point of view (Figure 3). The participants were instructed to bite repeatedly every 30-seconds, followed by 30-seconds of rest during 20 minutes. Pain intensity (NRS 0-10) and fatigue (Borg scale 6-20) were assessed. The microdialysis continued one hour after the tooth-clenching task. PPT was assessed before and after microdialysis. The STAI and PSS-14 questionnaires were assessed to measure the levels of anxiety and stress.



Figure 3. Illustration of the experimental tooth-clenching task that was performed in study IV during microdialysis (140-160 min). Visual feed-back was displayed on the bite-force transducer so that the participants could maintain a steady bite-force (50 % of their mean MVCF).

Statistics

Data was analyzed with SigmaPlot for Windows, version 11 (Systat Software Inc., Chicago, IL, USA in studies I-IV), SPSS software version 15.0 (SPSS Inc. Chicago, IL, USA in study III) and STATISTICA, StatSoft Dell Software version 12.0 (Round Rock, Texas USA in study IV). To test if the data was normally distributed, the Shapiro-Wilk's test was used (studies I- IV). In studies II-IV the data was not normally distributed and therefore non-parametric statistical analyzes were used. For the descriptive statistics the mean and standard deviation were used in study I, and median and IQR were used in studies II-IV. A power calculation was done in each study to include a sufficient number of participants in order to detect a statistically significant difference. For all studies a significance level of 5 % was set.

Changes in pain variables

In *study I*, parametric statistics were used and two-way mixed model ANOVA was used to analyze pain intensity after each injection and to compare differences between sides after pre-treatment with granisetron or placebo. Time was set as the repeated factor, and pain intensity (VAS) as the dependent factor. The Holm Sidak test was used for multiple comparisons and served as a post-hoc test.

In *study II*, the Wilcoxon test was used to analyze differences in pain variables after injections. ANOVA on ranks (Kruskall-Wallis test) or Mann-Whitney U-test was used to test the effect of the different SNPs on pain intensity, pain duration, pain area and PPT after HS injection and pre-treatment with granisetron.

In *study III*, The Mann-Whitney U-test was used to analyze differences in pain intensity between the HS and control side at the different time-points but also if there were any differences in the maximum pain intensity between sides. Spearman's correlation test, adjusted for multiple testing with Bonferroni correction, was used for analyzes of significant correlations between pain and the release of biomarkers.

In *study IV*, the Wilcoxon test was used to compare the time points 140 minutes (BL) and 160-220 minutes within each group. The Mann-Whitney U-test was used to analyze differences in the mean values (20-220 min) between groups, but also if there were any differences between groups at the time points 160 minutes (after tooth-clenching). In addition, the Mann-Whitney U-test was used to compare cytokine levels, pain intensity and fatigue (due to tooth-clenching) within and between groups. The Spearman's correlation test with Bonferroni correction for multiple testing, was used to analyze correlations between pain intensity, level of fatigue and cytokine levels.

In the thesis, ANOVA on ranks (Kruskall-Wallis test) was performed to investigate the differences in pain intensity, pain duration and pain area between the different experimental pain models. The Mann-Whitney U-test was used to analyze differences in pain intensity, pain duration and pain area after pre-treatment with granisetron or placebo.

Changes in pressure pain thresholds

In *study I*, one-way repeated measures ANOVA was used to test the significance of the changes in PPT. The PPT values after the injections were normalized to baseline, i.e. the relative changes (%) were used in the statistical analyzes.

In *study II*, the Wilcoxon test was used to analyze differences in PPT after injections. The values were normalized to the baseline value i.e. expressed in percent change. The mean values of the PPT (PPT_{mean}) at the different time points (5, 10, 15, 20, 25 and 30 min) were

calculated in order to compare the effect of different SNPs on changes of PPT. The PPT_{mean} value was normalized to the baseline value.

In *study III and IV*, the differences in PPT between sides before and after microdialysis was analyzed with unpaired t-test.

In the thesis, the t-test was used to analyze the relative changes (%) in PPT after pre-treatment with granisetron or placebo.

Changes in levels of biomarkers

In *study III*, the Friedman-test was used to analyze changes in biomarkers (5-HT, glutamate, lactate, pyruvate, glucose and glycerol) and pain levels over time on each side during microdialysis. A post-hoc test with Bonferroni correction was used when a significant difference was indicated by the Friedman-test.

In *study IV*, the changes in interstitial levels of cytokines over time was analyzed with repeated measures analysis of variance (RM ANOVA). However, since the Sphericity test was significant for all cytokines except IL-1 β , and attempts to transform data did not change this, non-parametric statistics were used. The Mann-Whitney U-test was used to analyze differences in cytokine levels between groups, by calculating and comparing the mean of all 11 dialysate samples (0-220 min). The Friedman-test analyzed changes in the levels of cytokines over time. Bonferroni correction was used to adjust for multiple testing.

Sex differences

In *study I*, unpaired t-test was used to test for significance between sexes in pain intensity, pain area and pain duration after acidic saline injections, but also in PPT at baseline (day 1) and before injections (day 3).

In *study II*, the Mann-Whitney U-test was used to analyze sex differences in pain variables, efficacy of granisetron and PPT after HS injections.

In *study III*, the Mann-Whitney U-test was used to test sex differences in pain intensity, pain duration, pain area and scoring of questionnaires (STAI and PSS-14). Unpaired t-test was used to test differences in PPT between sexes before and after microdialysis.

RESULTS AND DISCUSSION

Changes in pain variables

All experimental pain models respectively induced pain mainly localized to the masseter muscle, but the HS injections (*studies II and III*) caused pain with referral to adjacent regions such as the temporalis, teeth and forehead in some participants.

Acidic saline injections (*study I*) caused a low-moderate pain intensity for approximately 2 minutes. HS injections (*studies II and III*) evoked pain of moderate intensity during approximately 4-5 minutes. Tooth-clenching (*study IV*) increased pain and fatigue in TMD myalgia patients and increased the level of fatigue in healthy controls. There was a difference in pain intensity and fatigue before and after tooth-clenching between patients and controls (P 's < 0.001). HS injections caused higher pain intensity than acidic saline injections and experimental tooth-clenching, in healthy controls (P < 0.001), but in TMD myalgia patients, the pain intensity increased to the same magnitude as HS injection in healthy participants (Figure 4).

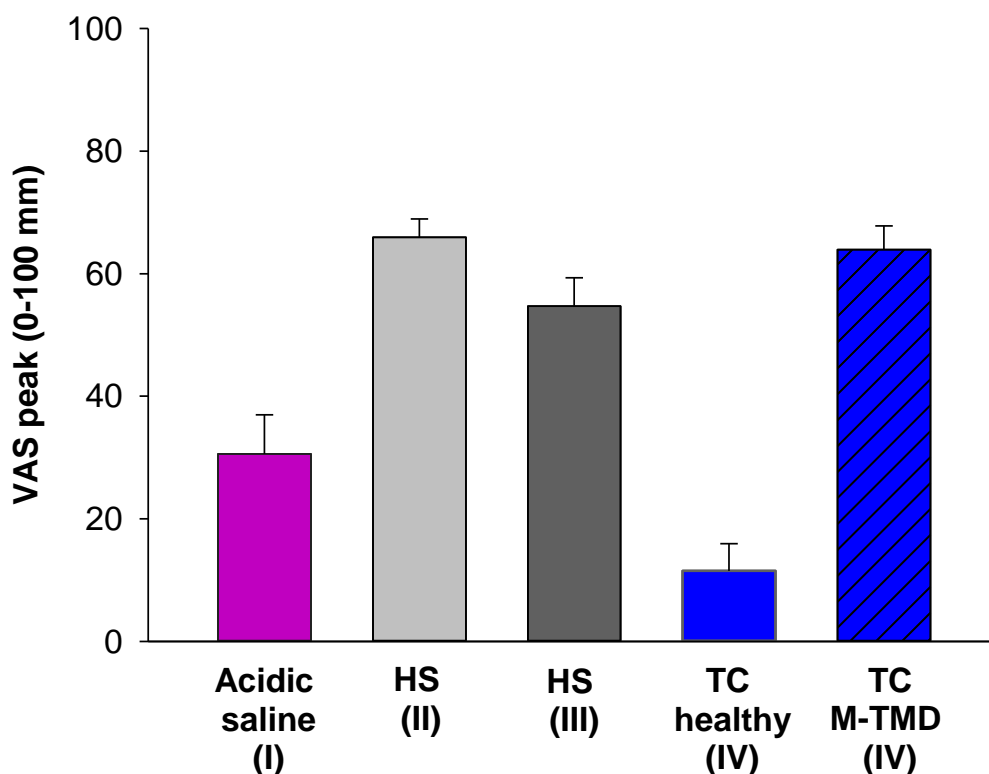


Figure 4. Graph showing the mean (SD) peak pain intensity (VAS 0-100 mm) in the masseter muscles of healthy participants and patients with TMD myalgia, in each experimental pain model. I = study I; II = study II; III = study III; IV= study IV. TC = Tooth-clenching.

The differences in pain duration and pain area between HS injections and acidic saline injections are shown in Figure 5.

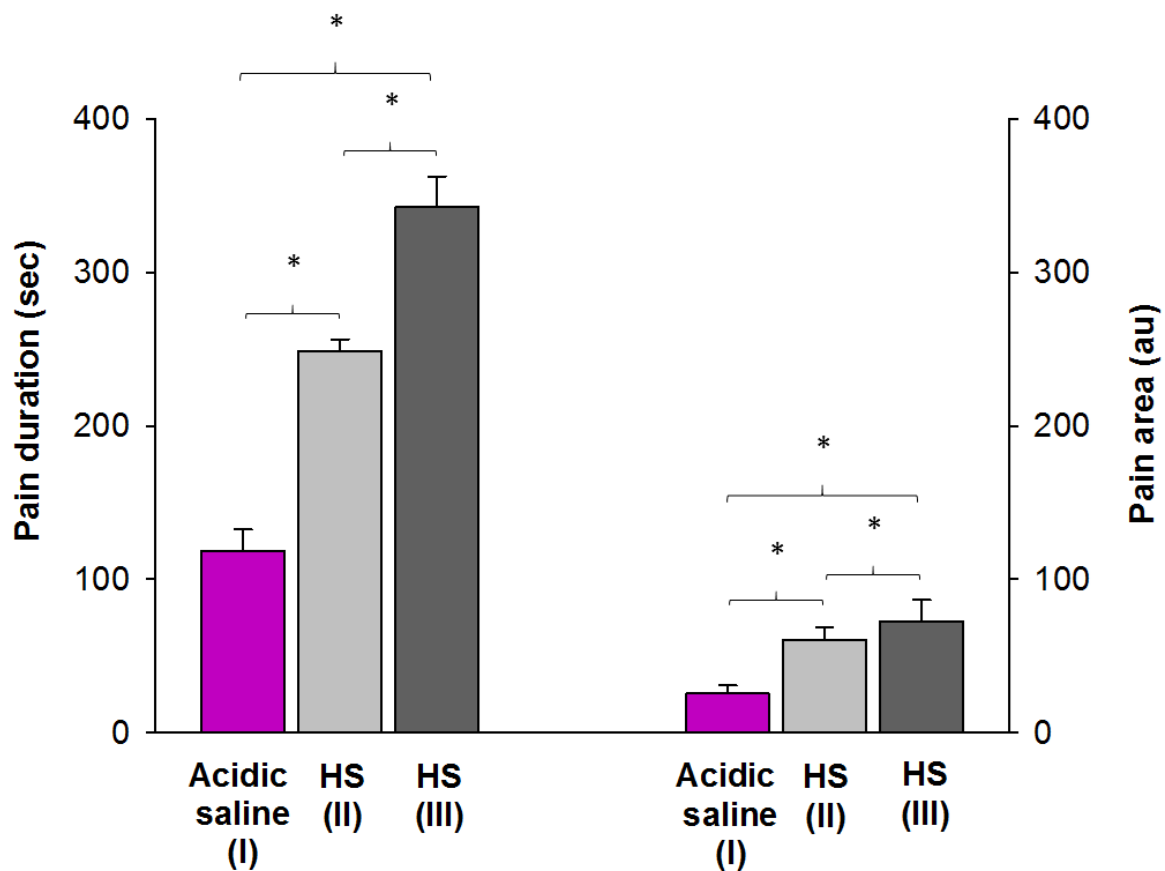


Figure 5. Graph showing the mean (SD) pain duration (sec) and painful area (au) in the masseter muscle of healthy participants after experimentally induced muscle pain with acidic saline and HS injections. The HS injections caused significantly longer pain duration ($P < 0.001$) and larger pain area ($P < 0.05$) compared to acidic saline injections. I = study I; II = study II; III = study III.

* = Significant differences between pain variables ($P < 0.05$).

The results illustrates that different experimental pain models induce muscle pain of different intensity, duration and area. Results from *studies II* and *III* show that HS injections caused a deep, diffuse pain with pain referral, in line with previous studies with no side effects (Graven-Nielsen, 2006). But, since it causes a short-lasting pain of a more acute character, another experimental pain model that mimics the clinical situation in a better way would be preferable.

In animal studies, repeated intramuscular injections of acidic saline caused a long-lasting allodynia with pain referral (Lund et al., 2010; Sluka et al., 2001). On the contrary, in another

study, repeated acidic saline injections in rodents did not cause long-lasting allodynia (Ambalavanar et al., 2007). In a human experimental study, a single injection of buffered acidic saline (pH 5.2) caused a mild to moderate pain with pain referral and mechanical allodynia lasting for 20 minutes (Frey Law et al., 2008).

With this in mind, it was relevant to investigate if the similar experimental pain model as used in animal studies, with repeated intramuscular acidic saline injections, would generate similar results in humans. However, results from *study I* showed that repeated intramuscular injections of acidic saline in the masseter muscle caused a short-lasting, low-moderate pain with no pain referral, thus was unsuccessful to better mimic the clinical condition. This is supported by a previous study where repeated injections of acidic saline in the masseter muscle failed to induce a long-lasting hyperalgesia (Castrillon et al., 2013). However, in contrast to the previous human study that used buffered acidic saline (Frey Law et al., 2008), these human studies used un-buffered acidic saline which could be one explanation for the contradictory results. Nevertheless, even though, an enhancement of the experimental pain model has been tested, using a buffered solution, it did not cause a long-lasting hyperalgesia in the masseter muscle (unpublished results). Furthermore, in a microdialysis study, acidic saline infusions did not increase the interstitial levels of algescic substances in the masseter muscle (Ernberg et al., 2013). Thus, repeated intramuscular acidic saline injections do not seem to be a valid experimental pain model for chronic muscle pain.

Excessive muscle work is another experimental pain model that has been used in several studies to investigate the pathophysiology behind musculoskeletal pain (Armstrong, 1984; Gerdle et al., 2008b; Larsson et al., 2008; Mengshoel et al., 1995; Miles and Clarkson, 1994; Strom et al., 2009). Since overload due to bruxism is suggested to be a risk factor for TMD, there are a number of studies examining different experimental tooth-clenching tasks and the effect on pain (Farella et al., 2010; Hedenberg-Magnusson et al., 2006; Torisu et al., 2007). In line with a previous study (Dawson et al., 2015), results from *study IV* show that tooth-clenching induced higher pain intensity and fatigue in patients with TMD myalgia than in healthy controls (P 's < 0.001). In patients, the pain intensity increased after tooth-clenching to a similar intensity as HS injections induced in healthy controls. However, only one healthy participant reported pain (NRS 1/10) after tooth-clenching. Similar findings, using the same tooth-clenching model, induced low levels of pain intensity in healthy participants (Dawson et al., 2013). In another study where experimental tooth-grinding was used, with a maximal voluntary occlusal force of 50 % for 9 sessions during 45 minutes, report significantly

increased soreness, but not pain, in the masseter muscle immediately after the experiment in ten healthy male participants (Arima et al., 2000). Other studies using different experimental tooth-grinding and tooth-clenching models show a similar pattern, where low levels of pain are induced in healthy participants immediately after the exercise and quickly disappears (Bowley and Gale, 1987; Scott and Lundeen, 1980; Svensson et al., 2001a).

Overall, the differences on pain characteristics between the experimental pain models used in this thesis, suggest that not all experimental pain models are adequate to use in healthy participants when studying chronic human muscle pain.

Effect on pain variables of granisetron

Results on pain intensity after pre-treatment with granisetron or placebo, followed by acidic saline or HS injections respectively are shown in Figure 6.

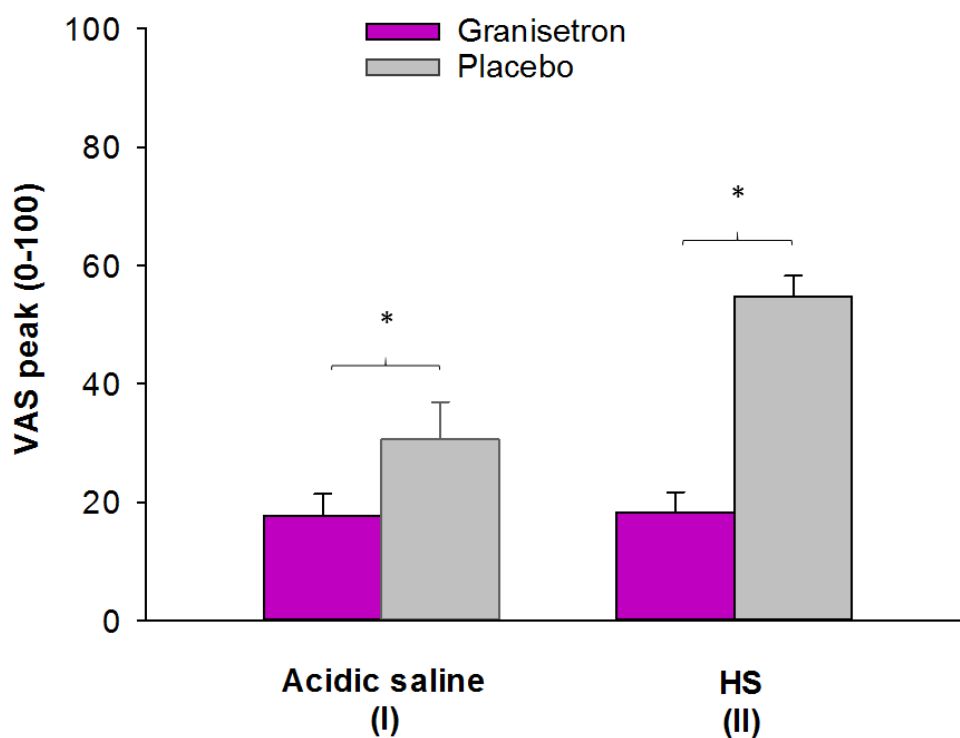


Figure 6. Graph showing the mean (SD) peak pain intensity (VAS 0-100 mm) in the masseter muscle of healthy participants after pre-treatment with granisetron or placebo and experimentally induced muscle pain with acidic saline or HS injections. The pain intensity was significantly lower on the side pre-treated with granisetron compared to placebo, both after acidic saline ($P < 0.05$) and HS injections ($P < 0.001$). I = study I; II = study II.

* = Significant differences between substances (granisetron and placebo).

The pain duration and pain area after pre-treatment with granisetron or placebo, followed by acidic saline or HS injections respectively are displayed in Figure 7.

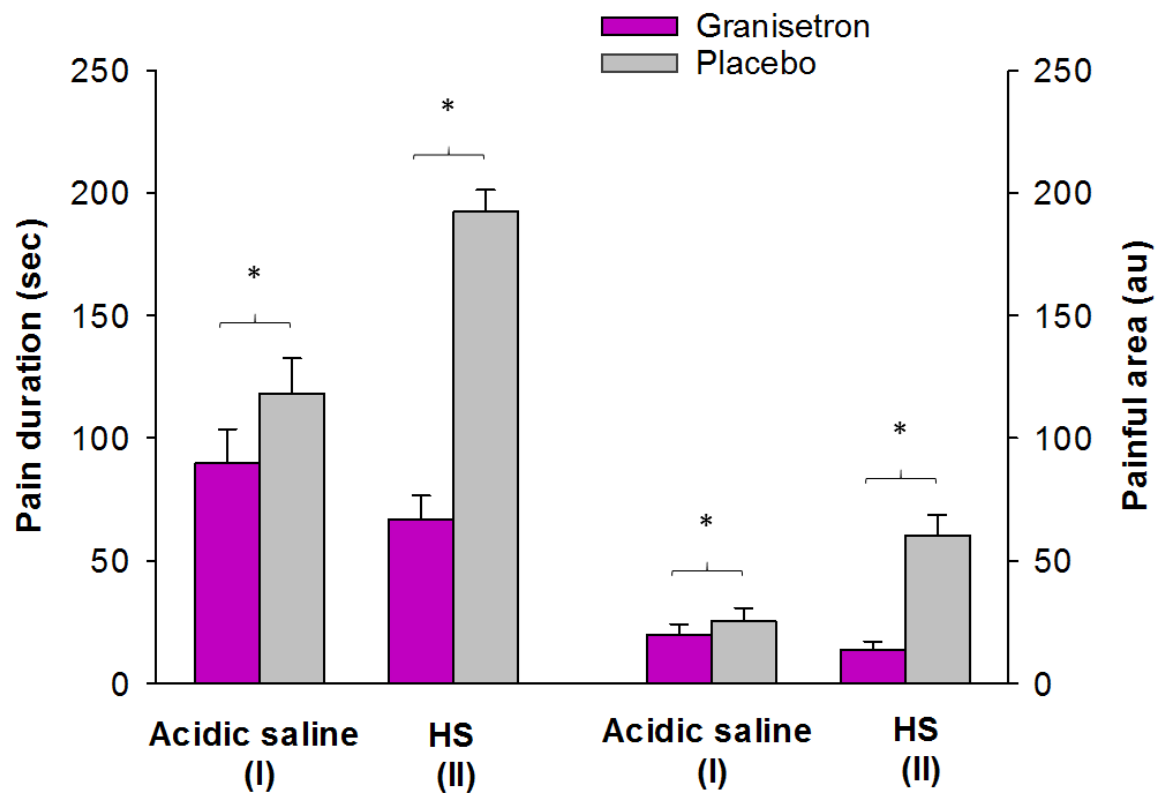


Figure 7. Graph showing the mean (SD) pain duration (sec) and painful area (au) in the masseter muscle of healthy participants after pre-treatment with granisetron or placebo and experimentally induced muscle pain with acidic saline or HS injections. The pain duration and pain area was significantly lower on the side pre-treated with granisetron compared to placebo, both after acidic saline ($P < 0.05$) and HS injections ($P < 0.001$). I = study I; II = study II.

* = Significant differences between substances (granisetron and placebo; $P < 0.05$).

The second HS injection induced less pain than the first ($P < 0.001$) which indicates that an activation of the diffuse noxious inhibitory control (DNIC) occurred. Since pain was induced simultaneously at both sides, the effect was the same, thus did not influence the results.

In accordance to previous studies, pre-treatment with granisetron reduced experimentally induced muscle pain (Christidis et al., 2008; Ernberg et al., 2000a), both by repeated acidic saline injections and HS injections. Several studies have shown a pain reducing effect of 5-HT₃-antagonist on other types of pain conditions such as neck pain, back pain and

fibromyalgia (Ettlin, 2004; Farber et al., 2001; Stratz and Muller, 2004). However, the pain reducing effect was greater after the HS injection compared to the acidic saline injections. Pre-treatment with granisetron caused 66 % lower pain than the control side after a HS injection, compared to 42 % lower pain after acidic saline injections. One explanation might be that the acidic saline injections caused lower levels of pain compared to the HS injection, and thus the possibility to reduce the pain intensity is less. Nevertheless, the pain intensity after acidic saline injections and granisetron was reduced to the same magnitude as after the HS injection and granisetron (Figure 7).

The variance in the pain reducing effect of granisetron and other 5-HT₃- antagonists, has also been shown in other studies (Christidis et al., 2007; Ernberg et al., 2000a; Muller and Stratz, 2004; Stratz et al., 2004). The reasons for this might be due to the usage of different 5-HT₃-antagonists, since granisetron has higher affinity to the 5-HT₃ receptor than tropisetron (Wong et al., 1995). Furthermore, the difference in methodology might also explain the variation in results. For example, two of the studies used repeated injections and were clinical (Ettlin, 2004; Stratz and Muller, 2004), whereas other studies were experimental and used a single injection of granisetron (Christidis et al., 2007; Voog et al., 2000).

The evoked muscle pain by the different experimental pain models in this thesis may be due to a direct or an indirect activation of the nociceptors (Cairns et al., 2003). A direct activation through the sodium channels, and indirectly by the increased levels of algescic biomarkers (Cairns et al., 2003). The positive effect of granisetron on pain variables can therefore be discussed. Previous studies have shown that HS increased levels of glutamate (Tegeder et al., 2002) and SP (Garland et al., 1995), and results from *study III* show an increased release of 5-HT. This could indicate that the pain reducing effect might partially be due to a block of the 5-HT₃ receptors. However, there was also a positive effect on pain variables after acidic saline injections. In a previous microdialysis study, painful infusions with acidic saline showed no increased levels of algescic biomarkers such as 5-HT and glutamate in the masseter muscle (Ernberg et al., 2013). Hence, the pain reducing effect of granisetron might also be that the sodium channels are affected (Kuryshev et al., 2000).

Changes in pressure pain threshold

The PPT at baseline and after each experiment are shown in Figure 8.

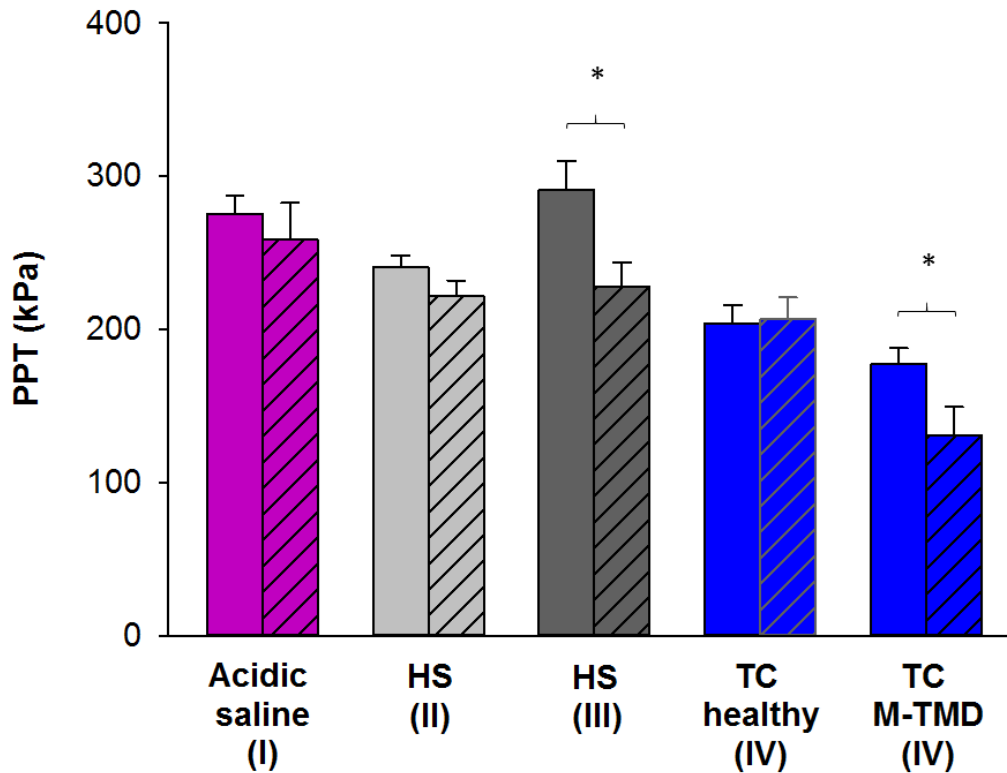


Figure 8. Graph showing the mean (SD) values of pressure pain thresholds (PPT; kPa) before and after each experiment. In studies III and IV (where microdialysis was performed), there was a significant difference before and after each experiment, in healthy controls (study III: $P < 0.011$) and in TMD myalgia patients (study IV: $P < 0.024$). I = study I; II = study II; III = study III; IV= study IV. TC = Tooth-clenching.

* = Significant change compared to baseline ($P < 0.05$).

After each experiment, PPT generally decreased but not consistently. In patients with TMD myalgia, the baseline values of PPT were lower than the control group ($P < 0.001$) and decreased significantly after tooth-clenching ($P < 0.024$). This was not surprising since pain on palpation is generally regarded as a key symptom of chronic muscle pain and has the strongest associations with TMD (Greenspan et al., 2011).

However, there are contradictory results regarding PPT after experimentally induced muscle pain. A few studies report reduced muscle PPT after HS injections while other studies report no effect (Graven-Nielsen, 2006). The various results regarding PPT in this thesis may be due to different modalities in pain stimulation by the experimental pain models, recruitment of the different groups and their previous pain experiences and the different methodology. For example, the pain intensity, pain duration and the pain spread varied after the experimental settings, which may have affected the outcome of the PPT. Yet, after a HS injection (*studies II and III*), PPT reduced significantly in *study III* but not in *study II*. Nevertheless, in *study III*, microdialysis was performed i.e. the trauma caused by the needle and probe inserted intramuscularly may have caused a sensitization in the muscle tissue, which possibly explains the reduced PPT.

Further, PPT decreased significantly compared to baseline after microdialysis, in healthy participants (*study III*) and in TMD myalgia patients, but not in the control group (*study IV*). Also in another microdialysis study, it was shown that PPT was decreased significantly in TMD myalgia patients. (Dawson et al., 2014). One explanation for the differences could be that there were two different study populations that vary in their baseline PPT. Another explanation could be that the number of healthy participants in *study IV* was too small (n=10).

The PPT did not increase after pre-treatment with granisetron (Figure 9). This was surprising since previous studies consistently have shown an increase of PPT after pre-treatment with granisetron, both locally and systemically (Christidis et al., 2008; Christidis et al., 2005; Christidis et al., 2007; Ernberg et al., 2003), although it looks like there was an increase of PPT in *study I* ($P = 0.243$). Yet, the different study populations such as their previous pain experiences, previous participation in other clinical studies (Sjolund and Persson, 2007) or different responses depending on female/male examiners must be taken into consideration (Levine and De Simone, 1991).

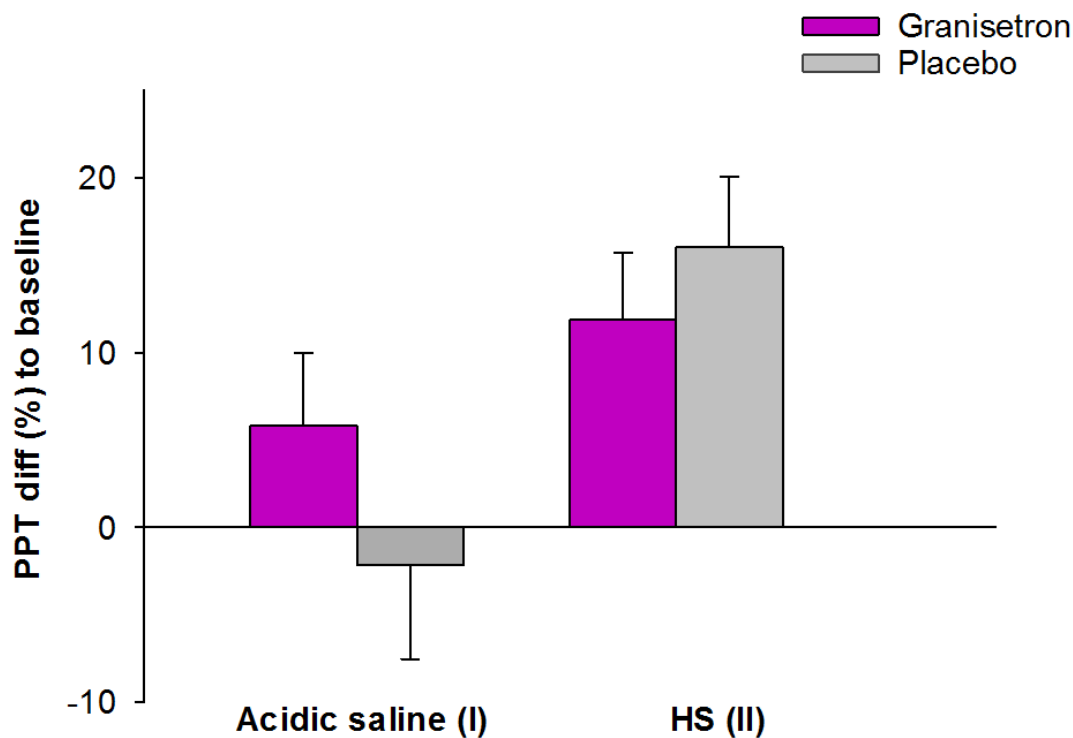


Figure 9. Graph showing the mean percentage differences of pressure pain thresholds (PPT; kPa) compared to baseline (0) and between substances, after pre-treatment with granisetron or placebo. There were no differences in the PPT between sides i.e. pre-treatment with granisetron did not affect the PPT. I = study I; II = study II.

There were no significant changes in the PPT over the reference point (the right index finger) in any of the studies, suggesting that no central sensitization occurred. Allodynia is generally regarded a sign of central sensitization (Woolf, 2011), but the lowered PPT in the masseter muscle after each experiment may be due to peripheral sensitization.

5-HT₃ polymorphisms and pain variables

In *study II*, participants were genotyped regarding the polymorphisms, rs1062613 and rs1176744 from the HTR3A/B genes. In the HTR3A gene, the distribution of the polymorphisms were as follows; 36 participants with the C/C genotype, 22 with C/C and 2 with T/T. Since only two participants had the T/T genotype, it was combined with the heterozygous genotype C/T and compared to the homozygous genotype C/C. In the HTR3B gene, the distribution were; 23 participants with the genotype A/A, 26 with A/C and 11 with C/C.

There were no differences in pain intensity, pain duration, pain area or PPT between the different HTR3 alleles. Also, there were no differences between the HTR3 alleles in the treatment effect of granisetron on pain intensity, pain duration, pain area and PPT. Overall, the main results show no direct association between the polymorphisms of the HTR3A/B genes respectively and experimental induced muscle pain by HS injection or the effect of granisetron.

A previous study showed that there are 358 genes thought to be involved in pain processing, but only a few have been associated with TMD namely; HTR2A, COMT, NR3C1, CAMK4, CHRM2, IFRD1, and GRK5 (Smith et al., 2011). Yet, none of the polymorphisms have shown an association with the risk of TMD onset, however several polymorphisms were associated with intermediate phenotypes shown to be predictive of TMD onset (Smith et al., 2013). Other studies have reported an association with polymorphisms in the HTR3A/B genes in a few pain conditions and psychiatric disorders (Hammer et al., 2009; Kapeller et al., 2008; Kilpatrick et al., 2011; Niesler et al., 2001; Yamada et al., 2006) and also healthy subjects with the SNP rs1176744, carrying the C genotype in the HTR3B gene showed an association to higher Pain Catastrophizing Scale scores. However, results from *study II*, show no association between the HTR3 genes and experimental muscle pain.

Other studies have shown that polymorphisms in HTR3A/B genes, influence the effect of the 5-HT₃ antagonist and the antidepressant response of the serotonin selective reuptake inhibitor (SSRI) paroxetine, by serving as predictors (Kato et al., 2006; Niesler et al., 2008). However, results from *study II* showed no direct association between the polymorphisms and the efficacy of granisetron. Yet, the sample size was relatively small which may have affected the results. Nevertheless, there were not even tendencies to a difference between the pain variables or the effect of granisetron and polymorphisms, suggesting that the results reported might reflect the true circumstances.

However, there are limited studies investigating the association between 5-HT polymorphisms and pain conditions and the effect of granisetron and various pharmaceuticals. Therefore, no further conclusions may be drawn.

Microdialysis and the levels of biomarkers

In *studies III* and *IV*, intramuscular microdialysis in the masseter muscle was performed to sample muscle biomarkers and investigate if changed levels occurred after experimentally induced muscle pain. The microdialysis technique has been used in several studies in order to study the local in vivo biochemistry of different tissues (Ernberg et al., 1999; Gerdle et al., 2008a; Gerdle et al., 2010; Rosendal et al., 2004b; Shah et al., 2005; Tegeder et al., 2002).

Study III

There were increased levels of 5-HT, glutamate and glycerol (P 's < 0.05) after a HS injection and there was a significant correlation between pain intensity and the levels of 5-HT and glycerol. Also, 5-HT, glutamate and glycerol changed significantly over time at the HS side (P < 0.05; Friedman test) but not at the control side. The other mediators lactate, pyruvate and glucose, did not differ over time or between sides (Figure 10).

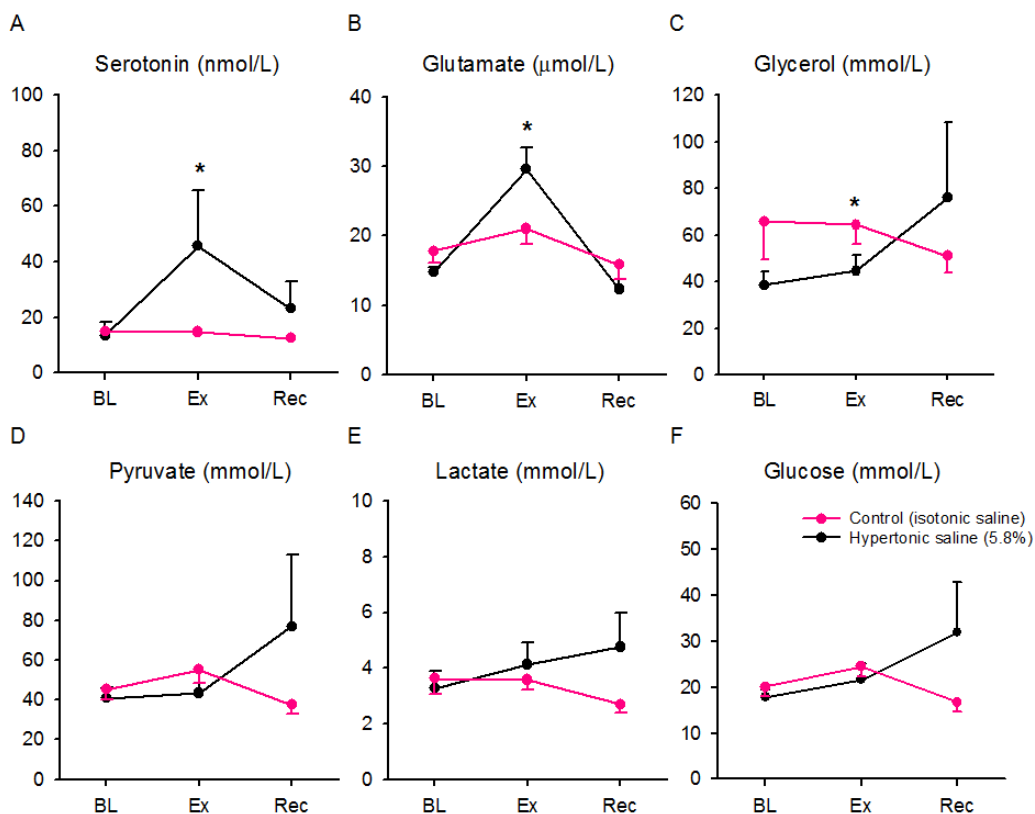


Figure 10. Graph showing the levels of biomarkers in the masseter muscle during the baseline (BL), experiment (Ex) and recovery (Rec). The biomarkers investigated were: serotonin (A), glutamate (B), glycerol (C), pyruvate (D), lactate (E) and glucose (F).

* = Significant differences between substances (P < 0.05).

The results are in line with previous studies showing increased levels of 5-HT in patients with TMD compared to healthy controls (Ernberg et al., 1999; Ghafouri et al., 2010; Shah et al., 2005). Also, a previous study showed that glutamate was released after a HS injection in the bicep muscle in healthy participants (Tegeder et al., 2002). Other studies reported no increased levels of 5-HT or glutamate in the masseter muscle after experimental tooth-clenching and acidic saline injections (Dawson et al., 2013; Ernberg et al., 2013). The reason for the different outcomes may be due to various experimental pain model that was used i.e. since lower pain intensity was evoked by experimental tooth-clenching and acidic saline injections compared to HS injections. This indicates that HS, which already is regarded as a valid experimental pain model for myalgia, also seem to be valid due to the release of 5-HT and glutamate.

There are a number of studies reporting increased levels of 5-HT and glutamate in trapezius and masseter myalgia with a positive correlation to muscle pain and tenderness (Castrillon et al., 2010; Ernberg et al., 1999; Flodgren et al., 2005; Ghafouri et al., 2010; Larsson et al., 2008; Rosendal et al., 2004b). Although these studies were performed by the same research group following similar study protocols, one can consider the findings reliable. This, since another research group has used a similar study protocol in respect of the duration of microdialysis, the solution of dialysate, the perfusion rate as well as recovery (Stahle and Borg, 2000).

The results from *study III* show a positive correlation between 5-HT and pain intensity which is in line with previous studies (Ernberg et al., 1999; Gerdle et al., 2008b), indicating that 5-HT plays an important role in muscle pain. Also, patients with TMD and trapezius myalgia showed a positive correlation between glutamate and pain (Castrillon et al., 2010; Rosendal et al., 2004b), however no such correlation was found in *study III*. This might be due to differences between clinical pain in TMD myalgia patients and experimentally induced muscle pain in healthy participants. Furthermore, glycerol correlated negatively to pain intensity. Glycerol has been suggested to be a useful marker of muscle damage after surgery (Hillered et al., 1998). However, since there are limited knowledge on the relationship between glycerol and pain, no certain conclusions can be drawn. Further studies are needed to estimate any possible relationship between glycerol and pain.

The blood flow did not alter between sides or after a HS injection, which is in line with a previous study using an experimental tooth-clenching task (Dawson et al., 2013). However, since we changed the dialysate vials every 20th minute, changes in the blood flow might have occurred during shorter periods of time, thus not have been detected. Since no measurable changes in the blood flow could be detected in *study III* and in previous studies (Dawson et al., 2013, 2015), the blood flow was not analyzed in *study IV*.

Furthermore, the questionnaires of psychological nature (STAI and PSS-14) indicate that the levels of anxiety and stress were within the normal range in both the men and women. A previous study suggest that the pain experience can be intensified by psychological aspects such as stress and anxiety (Melzack, 1980). However, no such interaction could be confirmed in this study.

Study IV

Several cytokines: IL-2, IL-4, IL-5, IL-10 and IFN- γ , could not be detected in a sufficient number of samples i.e. the LOD was below 50 %, therefore they were excluded from the statistical analysis, in line with previous study from our research group (Ernberg et al, personal communication). Another study has used similar approach but with less strict criteria (Chaturvedi et al., 2011).

There were higher levels of the pro-, and anti-inflammatory cytokines IL-6, IL-7, IL-8 and IL-13 in TMD myalgia patients compared to healthy controls during the entire microdialysis. After tooth-clenching, the cytokine levels of IL-6, IL-7, IL-8, IL-13 and TNF increased in TMD myalgia patients and IL-6 and IL-8 in controls (Figure 11), but there were no significant differences between the groups.

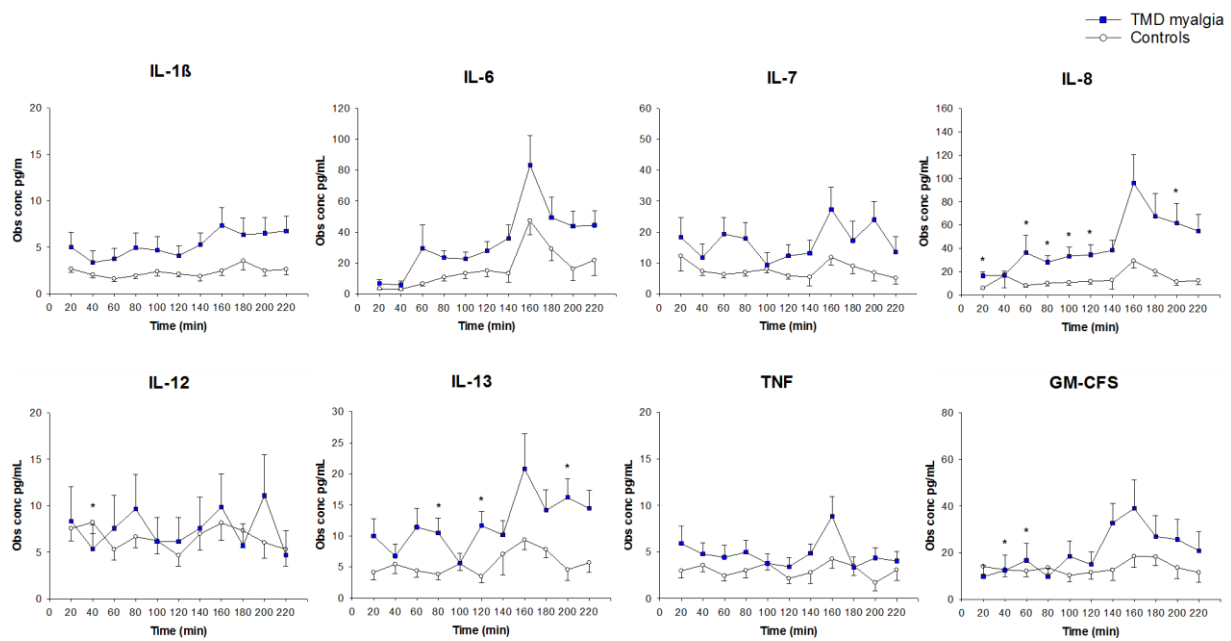


Figure 11. Graph showing the cytokine levels (mean and SEM) in the masseter muscle during microdialysis (0-220 min) in 20 women with TMD myalgia and 20 pain-free healthy women. After baseline (120-140 min), a 20-minute tooth-clenching task (140-160 min) was performed, followed by one hour of rest. The cytokines investigated were: IL-1 β , IL-6, IL-7, IL-8, IL-12, IL-13, TNF and GM-CFS.

* = Significant differences between groups ($P < 0.05$).

These findings are partly in line with other studies showing increased levels of IL-6 in the trapezius muscle in patients with WAD (Gerdle et al., 2008b) and IL-1 β , IL-6, IL-8 and TNF in myofascial trapezius trigger points (Shah et al., 2008; Shah et al., 2005). Further, recent results showed that the lipopolysaccharide (LPS) stimulated monocytes from patients with TMD, show an enhanced production of IL-1 β , TNF and IL-6 (King et al., 2016). This gives support to an involvement of peripheral mechanisms in chronic myalgia, and may implicate that patients with chronic pain constantly have their immune system switched on with higher levels of inflammatory mediators, leading to peripheral sensitization, which may drive the pain (Kidd and Urban, 2001).

In plasma, the cytokine levels seem to differ between patients with localized TMD and patients with widespread pain and TMD (Kosek et al., 2015; Park and Chung, 2016; Slade et al., 2011b). Although, cytokines in plasma were not analyzed in this study, previous study report elevated plasma levels of IL-1 β , IL-6, TNF and IL-10 in patients with localized TMD

(Park and Chung, 2016), whereas in patients with widespread pain and fibromyalgia, IL-8 was higher (Kosek et al., 2015; Slade et al., 2011b) and IL-10 lower compared to controls (Torgrimson-Ojerio et al., 2014). The reason for the diverse outcome may be due to differences in the balance between pro- and anti-inflammatory cytokines between localized TMD and widespread pain (Slade et al., 2011b), suggesting a more central pro-inflammatory state in widespread pain than in localized pain.

There were no significant differences in cytokine levels between patients and healthy controls after experimental tooth-clenching (160 min), although there was a tendency of higher levels of IL-8 in TMD myalgia patients in comparison to healthy controls (Mann-Whitney U-test; $P = 0.071$). A previous study partly supports this finding, where the levels of the pro-inflammatory cytokine IL-8 and pain intensity increased after a brief working task, in fibromyalgia patients and healthy controls, although no significant difference between groups were found (Christidis et al., 2015a). Further, several studies report higher levels of IL-8 in cerebrospinal fluid and serum in patients with fibromyalgia compared to controls (Gur et al., 2002; Kadetoff et al., 2012; Kosek et al., 2015).

Pain and fatigue increased in response to tooth-clenching, in accordance to a previous study (Dawson et al., 2015), but did not correlate to cytokine levels ($P > 0.102$; $r_s < 0.384$). This indicates that there is no direct cause-relation effect between pain and cytokine levels. Indeed, the increased pain might affect the release of other biomarkers (Ernberg et al., 1999; Rosendal et al., 2004b) or interactions between several other mechanisms (Svensson and Kumar, 2016). Yet, the balance and interaction between the pro- and anti-inflammatory cytokines as well as other inflammatory mediators needs to be considered before any further conclusions may be drawn.

One might argue that the results from *studies III* and *IV* might have been affected by the insertion of the needle, causing a trauma in the muscle. A previous study showed that there is an increased release of 5-HT after an insertion of a microdialysis probe in the muscle (Ernberg et al., 1999). Nevertheless, studies have shown that lactate, pyruvate and 5-HT return to baseline levels within 1 hour after insertion of the microdialysis catheters (Ernberg et al., 1999; Rosendal et al., 2004a). In order to avoid a false positive result, a stabilization period of 2 hours was therefore set (Gerdle et al., 2010; Ghafouri et al., 2010; Rosendal et al., 2004b). Also, one might claim whether the catheter was within the masseter muscle or not. However, when inserting the needle, a slight resistance is typically felt when penetrating the

muscle fascia, ensuring that the catheter was placed in the muscle in accordance to previous studies (Dawson et al., 2015; Ernberg et al., 2013).

Another issue, could be the use of local anaesthesia (*studies III and IV*) before the insertion of the needle, which might have affected the interstitial muscle levels of biomarkers. In *study III*, a local topical anaesthetic cream (EMLA) was applied for 30 minutes on the masseter muscle at the injection site. And in *study IV*, a subcutaneous local injection with Lidocaine (Xylocaine 20 mg /ml) was given. However, a previous study has showed that topical anaesthetic cream (EMLA) applied for 30 minutes on the forearm, did not penetrate deeper than 2.5 mm (Bjerring and Arendt-Nielsen, 1990). Hence, the local anaesthesia most probably was insufficient to influence the levels of biomarkers in the underlying masseter muscle in *study III*. Regarding the injection with Lidocaine, the injections were given subcutaneously, with caution not to penetrate the underlying muscle. However, one cannot exclude the possibility that the masseter muscle might have been affected by the local injection, which must be taken under consideration.

Sex differences

In *studies I-III*, sex differences were analyzed regarding pain intensity, pain duration, pain area and PPT. Also, in *studies I and II*, sex differences regarding the pain reducing effect of granisetron were evaluated. Furthermore, sex differences concerning biomarkers and 5-HT₃ polymorphisms were reported in *studies II and III*.

Experimentally induced muscle pain in the masseter muscles by acidic saline (*study I*) caused higher pain intensity, longer pain duration and larger pain area in women (P 's < 0.05). In *study II*, a HS injection caused higher pain intensity in women (P < 0.05), but the pain duration and pain area did not differ between sexes. However, in *study III*, a HS injection did not cause any sex differences in pain intensity, pain duration or pain area (Figure 12).

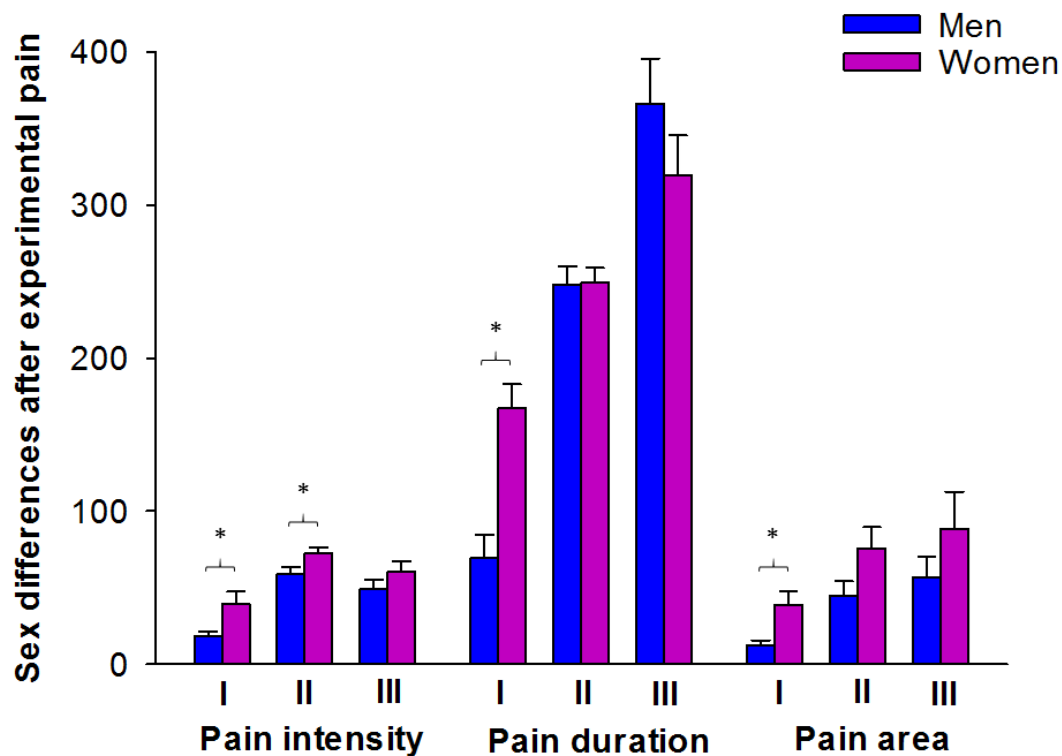


Figure 12. Graph showing the mean (SD) sex differences in pain intensity (0-100 mm), pain duration (sec) and painful area (au) after experimentally induced muscle pain in the masseter muscle in study I-III. I = study I; II = study II; III = study III.

* = Significant differences between sexes ($P < 0.05$).

In general, all experimental pain models caused more pain in women than men. This is in line with previous studies showing that women rate similar pain stimuli as more painful than men (Dao and LeResche, 2000), and are more sensitive to pain in different experimental settings such as chemical, electrical and thermal stimulus (Fillingim et al., 2009). For example, women experienced injections with glutamate and serotonin as more painful than men (Ernberg et al., 1999; Svensson et al., 2003) and reported larger pain spread after a HS injection in the masseter muscle (Christidis et al., 2008). In *study III*, there were no significant sex differences regarding pain intensity, pain duration or pain area which partly is supported by a previous study where no sex differences regarding pain intensity after a HS injection in the masseter muscle was reported (Christidis et al., 2008). However, although results from *study III* showed no significant differences, women in general reported higher pain intensity and larger pain area.

In *studies I* and *II*, the masseter muscle was pre-treated with granisetron. Granisetron had a pain reducing effect on both sexes, with lower pain intensity and lesser pain area. However,

men had a better effect of granisetron on pain variables in general. In *study I*, women had significantly longer pain duration than men ($P < 0.05$), and in *study II*, women reported higher pain intensity, longer pain duration and larger pain area (P 's < 0.05) compared to men (Figure 13).

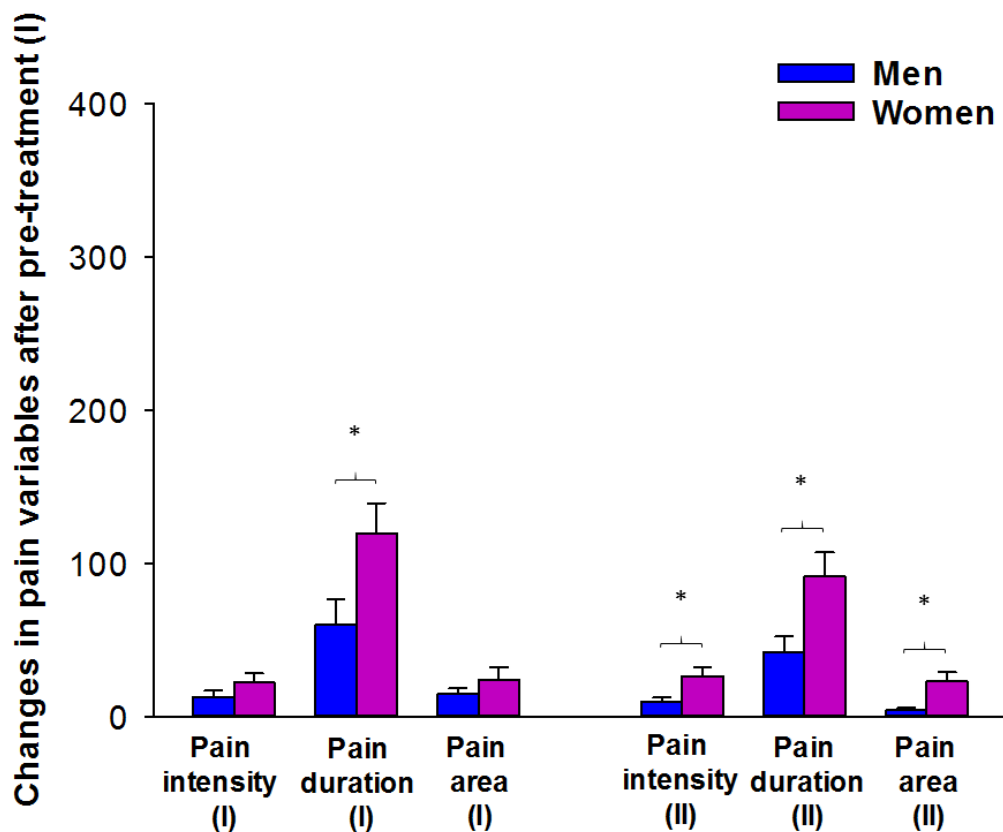


Figure 13. Graph showing the mean (SD) sex differences in pain intensity (0-100 mm), pain duration (sec) and painful area (au) after pre-treatment with granisetron in the masseter muscle in study I and II. I = study I; II = study II.

* = Significant differences between sexes ($P < 0.05$).

Other studies report no sex difference in pain intensity, pain duration or pain area in response to pre-treatment with granisetron (Christidis et al., 2008; Ernberg et al., 2000a). However, studies also report diverging results on the effect of opioid analgesia. For example, women seem to respond better to the analgesic effect of both μ and κ - opioid agonists compared to men (Fillingim and Gear, 2004; Gear et al., 1996; Niesters et al., 2010). On the other hand, in an animal study the same opioids did not show any sex differences in opioid potency and efficacy (Lomas and Picker, 2005). Overall, clinical human studies report a better analgesic effect of μ and κ - opioid agonists in women than men, while human experimental studies

show a greater effect of the μ -opioid agonists (Fillingim and Gear, 2004). The reason for the diverse results has been suggested to be due to multiple factors such as; hormonal influences, psychological factors, drug response, genetic factors and the balance of analgesic/anti-analgesic processes (Fillingim and Gear, 2004).

In general, the PPT over the masseter muscle was higher in men compared to women at baseline, which is in line with several studies (Christidis et al., 2005; Christidis et al., 2007; Ernberg et al., 1999; Ernberg et al., 2000b; Garcia et al., 2007; Okayasu et al., 2009). After experimentally induced muscle pain (*studies I-III*), the PPT did not differ significantly between sexes, although PPT seemed to be higher in men. Furthermore, there were no sex differences in PPT in response to granisetron (*studies I, II*). However, there are incongruent results regarding sex differences in response to granisetron in PPT. For example, one study showed that women had higher PPT than men after pre-treatment with granisetron and a painful injection with 5-HT (Ernberg et al., 2000a). Other studies report an increase of PPT in men after pre-treatment with granisetron (Christidis et al., 2008; Christidis et al., 2005). One reason for the diverse results might depend on if there was a female or male examiner (Levine and De Simone, 1991). Another explanation might be due to the study population i.e. their previous pain experiences and previous participation in other research studies (Sjolund and Persson, 2007).

In *study II*, there were no sex differences in the allele distribution in any of the 5-HT₃ polymorphisms. However, there were some sex-to-gene interactions in pain variables. After HS injection, women with the C/C genotype (HTR3A), had a larger pain area ($P = 0.015$), and in the A/C genotype (HTR3B), women had higher pain intensity than men ($P = 0.019$). After pre-treatment with granisetron, women with the T allele (HTR3A) had less reduction of pain intensity ($P = 0.041$) and pain area ($P = 0.005$), and women homozygous with the C allele (HTR3B) had less reduction of pain intensity ($P = 0.030$), pain duration ($P = 0.030$) and pain area ($P = 0.017$) compared to men.

A previous study have reported increased anxiety and amygdala responsiveness in IBS patients carrying the C/C genotype in the HTR3A gene (Kilpatrick et al., 2011). In another study, subjects with the C-allele in the HTR3B gene, were thought to be more sensitive to pain perception since a substitution of the nucleotides tyrosine to serine showed an increase response to 5-HT compared to tyrosine (tyr129) (Krzykowski et al., 2008). These results suggest the possibility that women carrying the C-allele of the HTR3A/B genes could be at

higher risk for increased anxiety and pain since pain and depression share similar pathways (Delgado, 2004). Furthermore, in similarity with earlier studies showing a sex difference in the effect of analgesics such as opioids (Fillingim and Gear, 2004; Gear et al., 1996; Niesters et al., 2010), there were also sex differences in response to the effect of granisetron. Genetic factors might be one of many factors contribution to sex differences.

Overall, the results show that women are more sensitive to different type of painful stimulus than men. Also, men responded better to the pain reducing effect of granisetron and had higher baseline PPT than women. The reason why women in general report the same painful stimuli as stronger than men can be discussed. Since pain is multifactorial, there are several factors thought to contribute to sex differences in pain response, for example biological, psychological and sociocultural factors. According to the well-recognized OPPERA study, the odds of developing TMD were reported three times higher in women compared to men, thus, viewed as a risk factor of TMD (Slade et al., 2011a). In another study, were sex differences was reported regarding the onset of TMD, more women than men developed TMD pain in their early adolescent, suggesting that sex hormones play an important role in the development of orofacial pain (TMD) (Nilsson et al., 2005). Furthermore, the pain thresholds in the masseter muscle varied during different phases of the menstrual cycle (Drobek et al., 2002). Also, psychological factors are suggested to affect the outcome. Previous study show that men report more anxiety than women, and that there is an association between high levels of anxiety and low pain thresholds (Frot et al., 2004).

In *study III*, the biomarker levels of 5-HT, glutamate, lactate, pyruvate, glucose or glycerol, did not differ between sexes at any time point. One could speculate if this result could be due to the small sample size. Yet, there were not even tendencies of sex differences in the release of biomarkers, suggesting that it might be a true finding. Since there are only a few microdialysis studies that report sex differences when examining the intramuscular levels of biomarkers, these findings cannot be confirmed. Hence, further studies that include both sexes are needed in order to compare biomarker levels between sexes.

Furthermore, there were no sex differences in the levels of anxiety (STAI) or stress (PSS-14). Previous studies have shown that more women than men are diagnosed with anxiety and mood disorders (Gater et al., 1998), and that psychological disorders can affect pain (Delgado, 2004). However, since we investigated a relatively small, young and healthy population group, it was not surprising that no sex difference was found.

GENERAL DISCUSSION

The riddle behind the complex mechanisms of chronic pain still remains. There are many pieces that needs to be put together to get a comprehensive view, for example both peripheral and central mechanisms most likely interact in the etiology of chronic pain (Scholz and Woolf, 2002). In this thesis however, the focus has been to highlight and investigate peripheral mechanisms that may contribute to the underlying factors behind the pathophysiology of TMD myalgia. Since it has been suggested that mechanical overloading and disturbed local blood flow may lead to local ischemia and peripheral release of algescic substances (“inflammatory soup”), which may induce and maintain muscle pain and thus be of importance for the pathogenesis of chronic myalgia (Mense, 1993, 2003), the emphasis has been on biomarkers in chronic and experimental human muscle pain.

The main findings were that granisetron had a pain reducing effect on experimentally induced muscle pain, with a generally better effect in men, but the 5-HT₃ polymorphisms did not influence the pain variables or the efficacy of granisetron in healthy individuals. Yet, there were sex differences in pain response and the pain reducing effect which seem to be influenced by the 5-HT₃ polymorphisms. Furthermore, experimentally evoked pain in the masseter muscle caused increased levels of the biomarkers; 5-HT, glutamate and glycerol in healthy pain-free participants, and 5-HT was positively correlation to pain intensity. Also, patients with TMD myalgia had constantly higher levels of pro- and anti-inflammatory cytokines compared to healthy controls, and several cytokines increased in response to tooth-clenching. However, there was no correlation between the cytokine levels and pain or fatigue.

Experimental pain models

The use of experimental pain models aim to mimic the clinical situation in order to study the pathophysiology behind chronic pain (Arendt-Nielsen et al., 2007). Experimental pain models should be standardized (Graven-Nielsen and Arendt-Nielsen, 2003; Graven-Nielsen et al., 2001; Le Bars et al., 2001). A standardized pain model reduces the variability in experimentally induced pain and increases reliability. Thus, a standardized pain model has a high validity if it shows similar results to the pain it will imitate (Arendt-Nielsen et al., 2007). Three experimental pain models, both endogenous and exogenous methods, have been used in this thesis namely; repeated acidic saline injections, HS injections and static tooth-clenching.

The results show that not all of the experimental pain models used in this thesis are ideal as models for chronic orofacial muscle pain, since there was a variability in pain variables. Repeated acidic saline injections caused low levels of muscle pain with no pain referral or mechanical hyperalgesia, and tooth-clenching induced low levels of muscle pain only in one single healthy participant, and was thus, inadequate to use on pain-free participants. HS on the other hand, induced high levels of muscle pain with pain referral. Nevertheless, the pain induced was more similar to acute pain.

Another criterion that may increase the validity of an experimental pain model, is to investigate whether the induced pain affects the levels of biomarkers in the tissue. Since patients with trapezius and masseter myalgia had increased levels of biomarkers (Ernberg et al., 1999; Ghafouri et al., 2010), it is reasonable to expect that experimental pain would show a similar pattern. A previous study showed that infusions with acidic saline into the masseter muscle did not cause the release of the biomarkers 5-HT and glutamate (Ernberg et al., 2013) in healthy participants, neither did static tooth-clenching, using the same methodology as in this thesis, cause higher masseter muscle levels of 5-HT and glutamate (Dawson et al., 2015). However, HS injection into the masseter muscle caused a significant release of 5-HT, glutamate and glycerol, and the level of 5-HT correlated to the pain intensity evoked (*study III*). This increases the validity of the HS injection as an adequate experimental pain model.

5-HT₃ polymorphisms and the efficacy of granisetron

In concordance to previous studies, experimentally induced muscle pain could be blocked by a single injection of the 5-HT₃-antagonist granisetron in *studies I* and *II* (Christidis et al., 2008; Ernberg et al., 2000a). Other 5-HT₃ receptor antagonists such as tropisetron and ondasetron also show a positive effect on clinical muscle pain conditions (Stratz et al., 2002; Stratz and Muller, 2004). The pain reducing effect of granisetron may not only be due to the blocking of the 5-HT₃ receptors, but also by the interaction with other substances in the chemical milieu. For example, one study showed that tropisetron inhibits PGE₂ (Seide et al., 2004) which have an immunomodulatory function on cytokines (Schneider et al., 2004). Also, another explanation to the pain reducing effect by the 5-HT₃ receptor antagonist may be that they affect sodium channels. The sodium channels are known to participate in peripheral pain and play a role in inflammatory pain (Cairns, 2009). Furthermore, another speculation

has been that polymorphisms in the serotonergic system may be involved in the pathophysiology of chronic muscle pain (Smith et al., 2011) and contribute to the analgesic effect of the 5-HT₃ antagonist granisetron. However, the pain reducing effect does not seem to be associated with the 5-HT₃ polymorphisms investigated in this thesis (*study II*), at least not experimentally induced muscle pain. Yet, there were sex differences in pain response, which nonetheless partly seem to be attributed to the 5-HT₃ polymorphisms investigated. A previous study showed that a combination of six polymorphisms was associated with greater odds of TMD (Slade et al., 2013). Therefore, the possibility that 5-HT polymorphisms could influence, predict or be a risk factor in developing chronic pain disorders such as chronic TMD myalgia cannot be excluded. Perhaps, several 5-HT polymorphisms systematically needs to be explored in further studies before any further conclusions can be drawn.

Biomarkers

Biomarkers, seem to play a role in biological processes such as pain modulation (Ptolemy and Rifai, 2010). Previous studies where the levels of biomarkers have been investigated, using the microdialysis technique, have shown that there are higher levels of biomarkers in different chronic myalgia conditions compared to healthy controls. For example, in patients with chronic trapezius myalgia, increased interstitial levels of 5-HT, glutamate, pyruvate and lactate were reported (Ghafouri et al., 2010; Larsson et al., 2008; Rosendal et al., 2004b) and 5-HT and glutamate correlated to pain (Gerdle et al., 2008b; Rosendal et al., 2004b). In other studies, increased masseter levels of 5-HT and glutamate were reported in patients with TMD myalgia (Castrillon et al., 2010; Ernberg et al., 1999) and 5-HT correlated to pain intensity (Ernberg et al., 1999). Furthermore, in *study IV*, the levels of pro- and anti-inflammatory cytokines were constantly higher in TMD myalgia patients compared to pain-free controls.

The higher levels of biomarkers in patients with chronic muscle pain compared to healthy controls, may support a previous suggestion that patients constantly have their immune system turned on (Kidd and Urban, 2001), supporting the hypothesis that peripheral mechanisms could be involved in the pathophysiology of chronic muscle pain. This imbalance of biomarkers, might trigger an inflammatory response, leading to peripheral sensitization that may in turn induce or maintain pain (Kidd and Urban, 2001). However, in *study IV*, there were also increased levels of anti-inflammatory cytokines, suggesting that they are produced in order to counterbalance this effect. Yet, not to a sufficient degree which might be due to a blunt response (King et al., 2016).

Nevertheless, the cytokine levels might have been affected by the insertion of the catheter causing a trauma in the muscle. Other studies have shown that the levels of 5-HT, lactate and pyruvate return to baseline within an hour after the insertion (Ernberg et al., 1999; Rosendal et al., 2004a), but it might possibly be different in regards to cytokines. A previous animal study showed that the plasma levels of cytokines in mice were elevated for 24 hours after skin damage compared to sham animals (Catania et al., 1999), and in a human study the cytokine levels were increased for 6 hours after the insertion of the microdialysis catheter into the vastus lateralis muscle in healthy male participants (Carson et al., 2015). Therefore, the stabilization period may be longer in patients with TMD myalgia compared to healthy controls which might have influenced the results.

The levels of cytokines did not correlate to the pain or fatigue evoked by the experimental tooth-clenching task, indicating that no direct cause-relation effect between cytokine levels and pain or fatigue can be confirmed. One explanation might be that other biomarkers are released in response to the inflammatory cascade, and involved in pain modulation (Ernberg et al., 1999; Rosendal et al., 2004b). Another reason could be that the interaction between several risk factors such as; pain intensity, trauma/overloading, psychological, genetics, sleep, comorbidities, demographics and lifestyle, could be combined in various ways and be categorized into low, moderate and high risk (Svensson and Kumar, 2016). Also, other mechanisms such as central sensitization might be involved in pain modulation (Kidd and Urban, 2001).

Furthermore, in patients with fibromyalgia or TMD with chronic widespread pain, there were higher plasma levels of pro-inflammatory cytokines (Kadetoff et al., 2012; Kosek et al., 2015; Slade et al., 2011b) and lower levels of an anti-inflammatory cytokine compared to healthy controls (Kadetoff et al., 2012). On the other hand, in patients with a more localized TMD, there were elevated levels of both pro- and anti-inflammatory cytokines (Park and Chung, 2016), suggesting that there is a difference in the biomarker balance between chronic widespread pain and localized muscle pain (Slade et al., 2011b), indicating a more central pro-inflammatory state in widespread pain. This could imply that peripheral sensitization may be of greater importance in localized myalgia compared to widespread pain.

Overall, the results from this thesis strengthen previous findings of peripheral involvement in chronic muscle pain (Castrillon et al., 2010; Ernberg et al., 1999; Gerdle et al., 2008b;

Ghafouri et al., 2010; Larsson et al., 2008; Rosendal et al., 2004b) since patients with masseter myalgia had elevated levels of biomarkers. This indicates that muscle inflammation could be involved in the pathophysiology of chronic masseter muscle pain. Still, a correlation between the levels of biomarkers and pain could not be confirmed. Therefore, other peripheral mediators and mechanisms, such as central sensitization might also be involved in the pathophysiology of chronic muscle pain.

Methodological considerations

Genotyping

A limitation that needs to be addressed was that the genotyping of the 5-HT₃ polymorphisms was performed after the experiment (*study II*), leading to uneven sized groups of participants with different genotypes, which complicated the statistical comparisons. Since only two participants had the genotype T/T (HTR3A), they were combined with the C/T group and compared to the homozygous C/C group, in line with a previous study (Kilpatrick et al., 2011). In order to avoid this in future studies, genotyping could preferably be performed beforehand, in accordance to a previous study (Horjales-Araujo et al., 2013). Furthermore, another factor that can be taken under consideration, is to have a larger study population, which will improve the power of the statistical analyzes.

Microdialysis

A strength was that 13 cytokines, both pro- and anti-inflammatory were analyzed in one panel so that differences in their pattern during the microdialysis could be compared. Even though several cytokines were under LOD and had to be excluded, the most common cytokines could still be detected. There are a number of factors that can affect the dialysate levels namely; a) the flow-rate b) the diffusion-rate through the tissue c) the area and weight cut-off of the dialysis membrane, and d) the composition of the perfusate (Gerdle et al., 2014). In future studies, the composition of the perfusate could be modified, maybe by adding a colloid to the ringer-solution, and thus perhaps detect more cytokines (Helmy et al., 2009). Furthermore, most microdialysis studies are performed in women, due to the higher prevalence of chronic myalgia conditions in women. Hence, in *study IV*, only women were included. In future studies men could also be included to be able to compare mediator levels between sexes.

Phase of the menstrual cycle

One factor that can be view as a limitation was that the phase of the menstrual cycle and the use of contraceptives were not taken into consideration. A previous study show that pain intensity varies during the menstrual cycle due to the levels of hormones (LeResche et al., 2003). Another study however, showed that the influence of the estrogen levels are contradictory (Warren and Fried, 2001), and most likely women participated in this project were in difference phases of the menstrual cycle, therefore, this aspect should merely have very limited or no impact on the results. However, this aspect probably has no major importance since it has been shown that the intra-individual variability in pain response is greater than the influence of estrogen (Sherman and Leresche, 2006).

Hypotheses addressed

The following hypotheses were addressed in this thesis;

Study I:

The hypotheses in *study I* were that 1) muscle pain induced by two repeated acidic saline injections into the masseter muscle of healthy volunteers can be blocked by the 5-HT₃ antagonist granisetron and 2) the pain-reducing effect by granisetron is better in men than in women.

The results support the first hypothesis since muscle pain induced by two repeated acidic saline injections into the masseter muscle of healthy participants were blocked by the 5-HT₃ antagonist granisetron. However, results did not support the second hypothesis since the pain reducing effect by granisetron on peak pain and pain area was significant for both sexes, and the effect on pain duration was significant only in women.

Study II:

The hypotheses in *study II* were that 1) polymorphisms in the serotonergic system are of importance for pain transmission and for the efficacy of the 5-HT₃ antagonist granisetron in experimentally induced muscle pain and 2) there are sex differences due to specific HTR3A/B genotypes in pain responses after experimentally evoked muscle pain and the analgesic effects of granisetron.

The results could not confirm the first hypothesis since results show that the 5-HT₃ polymorphisms did not seem to directly influence pain perception or the efficacy of granisetron for the group as a whole. However, results may support the second hypothesis, since there were some sex-to-gene interactions in pain response and in the effect of granisetron.

Study III:

The hypotheses in *study III* were that 1) muscle pain induced by HS in the masseter muscle causes a significant release of 5-HT, glutamate, lactate, pyruvate, glucose and glycerol and 2) the release of these muscle biomarkers are higher in women than in men.

The results partly support the first hypothesis, since there were increased levels of 5-HT, glutamate and glycerol after a HS injection and a significant correlation between pain intensity and the levels of 5-HT and glycerol. However, results did not support the second hypothesis, since there were no sex differences in biomarkers after HS injection.

Study IV:

The hypotheses in *study IV* were that 1) the levels of pro- and anti-inflammatory cytokines are significantly higher in patients with TMD myalgia both at rest and after a repetitive tooth-clenching task and 2) the release of cytokines are correlated with pain intensity and fatigue.

The results partly support the first hypothesis since TMD myalgia patients had significantly higher levels of the pro- and anti-inflammatory cytokines IL-6, IL-7, IL-8 and IL-13 during the entire microdialysis compared to healthy controls. After tooth-clenching, the pro- and anti-inflammatory cytokines IL-6, IL-7, IL-8, IL-13 and TNF increased in TMD myalgia patients and IL-6 and IL-8 in healthy controls, but without any differences between the groups. Further, the results did not confirm the second hypothesis since there was no correlation between the cytokine levels and pain intensity and fatigue.

CONCLUSIONS

The overall conclusions are that granisetron had a pain reducing effect on experimentally induced muscle pain, with a better effect in men in general. None of the 5-HT₃ polymorphisms appeared to influence the experimentally evoked muscle pain or the positive effect of granisetron.

Further, the levels of the biomarkers 5-HT, glutamate and glycerol increased after experimentally induced muscle pain in the masticatory muscles. In addition, TMD myalgia patients had higher levels of the pro- and anti-inflammatory cytokines IL-6, IL-7, IL-8 and IL-13 compared to healthy controls. After tooth-clenching, the levels of IL-6, IL-7, IL-8, IL-13 and TNF increased in patients, and IL-6 and IL-8 increased in controls.

Clinical implications and future research

Since the main aim of this thesis has been to focus on peripheral mechanisms behind chronic muscle pain, hence basic science, no direct clinical implication may be drawn. Nevertheless, basic science is essential in order to increase our understanding and broaden our knowledge with the purpose to improve the clinical approach, diagnostics and treatments.

Our findings have highlighted the importance of the serotonergic system and its effect on pain variables and sex differences. Future studies are needed to systemically explore several 5-HT polymorphisms in patients with chronic myalgia in order to better understand if and how they influence pain perception. Also, our results have shown that experimental pain causes increased levels of some peripheral biomarkers, but also that TMD myalgia patients have higher levels of some biomarkers compared to healthy controls, suggesting that chronic myalgia may be driven or maintained by muscle inflammation. However, more studies are needed to further explore other peripheral biomarkers and mechanisms such as central sensitization.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Studier har visat att en stor del av den svenska befolkningen lider av kronisk muskelsmärta. Omkring 40 % har smärtan lokaliserad till ansiktet och käkarna. En majoritet av dessa personer är kvinnor. Förutom det individuella lidandet är det ett stort problem för samhället med ökad sjukfrånvaro och sjukvårdskostnader som följd. Än så länge är inte orsakerna och mekanismerna som ligger bakom långvarig muskelsmärta fullständigt utredda. Vi vet inte heller varför kvinnor drabbas i högre utsträckning än män. Ökad kunskap om mekanismerna och orsakerna bakom denna muskelsmärta kommer att hjälpa oss förbättra diagnostiken, och medverka till nya behandlingsmetoder. Detta kan i sin tur på sikt leda till minskat behov av sjukvårdsresurser och vårdkostnader och inte minst, leda till minskat lidande för den enskilda individen.

Huvudsymptomen vid långvarig smärta lokaliserad till käkmuskulaturen och/eller käkleden är lokal smärta och ömhet s.k. myalgi, ofta förenat med nedsatt gapförmåga, huvudvärk och nackvärk. Olika teorier bakom långvarig muskelsmärta är att psykosociala faktorer i kombination med stressrelaterad tandgnissling och tandpressning (bruxism) är bidragande faktorer. Vid bruxism överbelastas musklerna med syrebrist till följd av otillräcklig blodcirkulation vilket bidrar till muskelinflammation. Vid en inflammation frisätts olika ämnen såsom serotonin (5-HT), glutamat och olika typer av cytokiner som förmodligen bidrar till smärtan genom att binda till särskilda mottagarmolekyler (receptorer). Det är inte osannolikt att liknande mekanismer även bidrar vid muskulära smärttillstånd i andra delar av kroppen. Sannolikt påverkas dessutom denna av stress- och könshormoner vilket skulle kunna vara en bidragande förklaring till att fler kvinnor än män drabbas av långvarig muskelsmärta.

Tidigare forskning har visat att 5-HT är en viktig substans för smärtfortledning vid långvarig muskelsmärta och att läkemedel som blockerar 5-HT har smärthämmande effekter. Det är dock en stor skillnad i effekt mellan olika individer. En förklaring är att varianter i vissa gener påverkar smärtskänsligheten och kan bidra till skillnaden i effekt av dessa läkemedel. På senare år har genetiska faktorer bakom olika sjukdomar fått stor uppmärksamhet. Även om ingen enskild gen har visats kunna orsaka långvarig muskelsmärta så har flera genetiska förändringar (polymorfismer) visats vara vanligare hos patienter med långvarig muskelsmärta än hos befolkningen i övrigt. Intressant är att polymorfismer i generna HTR3A/B har visat sig ha samband med depressionstillstånd. Eftersom långvarig muskelsmärta ofta är förenat med

depression skulle de också kunna påverka smärtkänsligheten och effekten av sådana läkemedel.

Det övergripande syftet med denna avhandling är att öka kunskapen avseende patofysiologin (orsaksmekanismerna) vid käkmuskelsmäta, för att därigenom kunna förbättra diagnostiken, och upprätta ett mer rationellt omhändertagande. Avhandlingen syftar till att med hjälp av experimentella smärtmodeller undersöka förhållandet mellan smärtvariabler och frisättning av olika smärtframkallande ämnen (5-HT, glutamat, och cytokiner). Dessutom undersöktes vissa polymorfismer i generna HTR3A/B och deras påverkan på experimentellt framkallad muskelsmäta och effekten av behandling med ett serotoninblockerande läkemedel (granisetron). Friska frivilliga försökspersoner, på vilka man framkallar experimentell muskelsmäta, samt patienter med käkmuskelsmäta deltog i experimenten.

I projektet användes olika experimentella smärtmodeller för att imitera/likna en långvarig muskelsmäta. I dagsläget finns ingen optimal experimentell smärtmodell som till fullo liknar ett kroniskt muskulärt smärttillstånd. Den smärtmodell som är mest validerad är injektion av hyperten koksalt, dock framkallar den en akut, skarp och kraftig smärta. Eftersom hyperten koksalt mer liknar en akut smärta skulle det vara fördelaktigt och eftersträvänsvärt att ha en smärtmodell som efterliknar den kliniska långvariga smärtan mer korrekt. I detta projekt har vi använt oss av tre olika smärtmodeller: a) upprepade injektioner med surgjord koksalt b) injektion med hyperten koksalt och c) upprepad statisk tandpressningsövning.

I den första studien använde vi oss av två upprepade injektioner av surgjord koksalt i käkmuskeln (massetermuskeln), då denna experimentella smärtmodell har visat sig vara framgångsrik i djurstudier där man lyckats efterlikna en mer långvarig smärta med en ökad smärtkänslighet 30 dagar efter den sista injektionen. I *studie I* undersökte vi därmed effekten av granisetron på den experimentellt framkallade muskelsmärtan samt om det förelåg några könsskillnader. Resultatet visade att granisetron hade en smärtreducerande effekt på den experimentellt framkallade muskelsmärtan genom upprepade injektioner med surgjord koksalt. Den surgjorda koksallösningen framkallade dock endast en mild lokal smärta i massetermuskeln utan någon ökad smärtkänslighet efter injektionerna. Trots att man i andra studier har försökt att modifiera smärtmodellen genom att öka injektionsvolymen samt använda sig av en buffrad surgjord koksallösning, har den inte visat sig vara lika framgångsrik som i djurstudier. Därmed, bedömdes den inte som optimal att använda i de övriga studierna.

I *studie II* användes injektioner med hyperton koksalt i massetermuskeln för att framkalla smärta. Blodprover togs för att analysera DNA och därmed titta på vilken roll polymorfismerna (rs1062613, rs1176744) i generna HTR3A/B har i förhållande till framkallad muskelsmärta i massetermuskeln och vilken effekt dessa har på läkemedlet granisetron. Resultaten visade att ingen av dessa polymorfismer påverkade den experimentellt framkallade muskelsmärtan i massetermuskeln eller effekten av granisetron på smärtvariabler generellt i hela gruppen, men det fanns vissa könsskillnader i smärtvariablerna som förefaller vara påverkade av genotyper.

I *studie III* användes återigen injektioner av hyperton koksalt i massetermuskeln för att framkalla smärta och undersöka om det ledde till en frisättning av vissa biomarkörer (5-HT, glutamat, laktat, pyruvat, glukos och glycerol), och om det fanns några könsskillnader i detta avseende. Vi använde oss av en så kallad mikrodialysteknik (teknik för att separera små molekyler från stora i en vätska med hjälp av diffusion), för att analysera vilka ämnen som frisätts i massetermuskeln vid smärta. Denna teknik användes också i *studie IV*. Resultaten visade att 5-HT, glutamat och glycerol ökade efter en smärtsam injektion med hyperton koksalt i massetermuskeln, men utan några könsskillnader. Eftersom förhöjda nivåer av 5-HT och glutamat tidigare har rapporterats vid långvarig muskelsmärta, stärker detta validiteten för den experimentella smärtmodellen.

I *studie IV* användes en 20-minuters statisk tandpressningsövning för att framkalla smärta i massetermuskeln. Vissa biomarkörer (cytokiner) undersöktes, dess svar på experimentell tandpressning och förhållande till smärta, trötthet och psykisk ohälsa hos patienter med långvarig muskelsmärta från massetermuskeln. Resultaten visade att nivåerna av vissa cytokiner (IL-6, IL-7, IL-8 och IL-13) är förhöjda i massetermuskeln hos patienter med långvarig muskelsmärta och att det ökade som svar på tandpressning. Tandpressningen ökade käkmuskelsmärtan och tröttheten, men utan någon korrelation till de ökade cytokinnivåerna. Den statiska tandpressningsövningen framkallade endast en låg smärta hos en enstaka frisk försöksperson, sålunda är denna smärtmodell kanske inte helt ideal för att använda på smärtfria försökspersoner.

Sammanfattningsvis tyder resultaten från denna avhandling på att granisetron visade sig ha en smärtreducerande effekt på den experimentellt framkallade muskelsmärtan i massetermuskeln, men inga av de undersökta 5-HT₃ polymorfismerna verkade påverka den

framkallade muskelsmärtan eller den positiva effekten av granisetron hos friska individer. Likväl fanns det könsskillnader i smärta och dess effekt på granisetron, vilka tycks vara påverkade av polymorfismerna. I och med detta, kan man inte helt utesluta att olika genvarianter i det serotonerga systemet kan påverka, förutsäga eller vara en riskfaktor för att utveckla kronisk muskelsmärta. Ytterligare forskning behövs dock för att systematiskt undersöka flera genvarianter för att dra några definitiva slutsatser.

Ytterligare resultat visade att vissa biomarkörer ökar vid experimentellt framkallad muskelsmärta i massetermuskeln. Dessutom hade patienter med långvarig muskelsmärta i massetermuskeln en konstant förhöjd nivå av inflammationssubstanser (cytokiner) vilka ökade som svar på tandpressning. Detta indikerar att en eventuell muskelinflammation skulle kunna vara inblandad i patofysiologin bakom kronisk käkmuskelsmärta.

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REFERENCES

- Ahn, J., and Ehrenpreis, E.D. (2002). Emerging treatments for irritable bowel syndrome. *Expert Opin Pharmacother* 3, 9-21.
- Alwi, Z.B. (2005). The Use of SNPs in Pharmacogenomics Studies. *The Malaysian journal of medical sciences : MJMS* 12, 4-12.
- Ambalavanar, R., Yallampalli, C., Yallampalli, U., and Dessem, D. (2007). Injection of adjuvant but not acidic saline into craniofacial muscle evokes nociceptive behaviors and neuropeptide expression. *Neuroscience* 149, 650-659.
- Arendt-Nielsen, L., Curatolo, M., and Drewes, A. (2007). Human experimental pain models in drug development: translational pain research. *Current opinion in investigational drugs* (London, England : 2000) 8, 41-53.
- Arima, T., Svensson, P., and Arendt-Nielsen, L. (2000). Capsaicin-induced muscle hyperalgesia in the exercised and non-exercised human masseter muscle. *J Orofac Pain* 14, 213-223.
- Armstrong, R.B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Med Sci Sports Exerc* 16, 529-538.
- Babenko, V., Graven-Nielsen, T., Svensson, P., Drewes, A.M., Jensen, T.S., and Arendt-Nielsen, L. (1999). Experimental human muscle pain induced by intramuscular injections of bradykinin, serotonin, and substance P. *Eur J Pain* 3, 93-102.
- Barr, A.E., and Barbe, M.F. (2002). Pathophysiological tissue changes associated with repetitive movement: a review of the evidence. *Phys Ther* 82, 173-187.
- Barton, B.E. (1997). IL-6: insights into novel biological activities. *Clin Immunol Immunopathol* 85, 16-20.
- Bennett, G.J. (2012). What is spontaneous pain and who has it? *J Pain* 13, 921-929.
- Benoliel, R., Kahn, J., and Eliav, E. (2012). Peripheral painful traumatic trigeminal neuropathies. *Oral Dis* 18, 317-332.
- Bickel, M. (1993). The role of interleukin-8 in inflammation and mechanisms of regulation. *J Periodontol* 64, 456-460.
- Binshtok, A.M., Wang, H., Zimmermann, K., Amaya, F., Vardeh, D., Shi, L., Brenner, G.J., Ji, R.R., Bean, B.P., Woolf, C.J., and Samad, T.A. (2008). Nociceptors are interleukin-1beta sensors. *J Neurosci* 28, 14062-14073.
- Bjerring, P., and Arendt-Nielsen, L. (1990). Depth and duration of skin analgesia to needle insertion after topical application of EMLA cream. *Br J Anaesth* 64, 173-177.
- Bowley, J.F., and Gale, E.N. (1987). Experimental masticatory muscle pain. *J Dent Res* 66, 1765-1769.
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., and Gallacher, D. (2006). Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. *European journal of pain* (London, England) 10, 287-333.
- Cairns, B.E. (2009). *Peripheral Receptor Targets for Analgesia. Novel Approaches to Pain Management*, Vol First (New Jersey, United States: Wiley).

Cairns, B.E., Svensson, P., Wang, K., Hupfeld, S., Graven-Nielsen, T., Sessle, B.J., Berde, C.B., and Arendt-Nielsen, L. (2003). Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. *Journal of neurophysiology* 90, 2098-2105.

Carson, B.P., McCormack, W.G., Conway, C., Cooke, J., Saunders, J., O'Connor, W.T., and Jakeman, P.M. (2015). An in vivo microdialysis characterization of the transient changes in the interstitial dialysate concentration of metabolites and cytokines in human skeletal muscle in response to insertion of a microdialysis probe. *Cytokine* 71, 327-333.

Castrillon, E.E., Cairns, B., List, T., Svensson, P., and Ernberg, M. (2013). Acidic saline-induced pain as a model for experimental masseter myalgia in healthy subjects. *European journal of pain* (London, England).

Castrillon, E.E., Ernberg, M., Cairns, B.E., Wang, K., Sessle, B.J., Arendt-Nielsen, L., and Svensson, P. (2010). Interstitial glutamate concentration is elevated in the masseter muscle of myofascial temporomandibular disorder patients. *Journal of orofacial pain* 24, 350-360.

Catania, R.A., Schwacha, M.G., Cioffi, W.G., Bland, K.I., and Chaudry, I.H. (1999). Does uninjured skin release proinflammatory cytokines following trauma and hemorrhage? *Archives of surgery* 134, 368-373; discussion 373-364.

Chaturvedi, A.K., Kemp, T.J., Pfeiffer, R.M., Biancotto, A., Williams, M., Munuo, S., Purdue, M.P., Hsing, A.W., Pinto, L., McCoy, J.P., and Hildesheim, A. (2011). Evaluation of multiplexed cytokine and inflammation marker measurements: a methodologic study. *Cancer Epidemiol Biomarkers Prev* 20, 1902-1911.

Christidis, N., Ghafouri, B., Larsson, A., Palstam, A., Mannerkorpi, K., Bileviciute-Ljungar, I., Lofgren, M., Bjersing, J., Kosek, E., Gerdle, B., and Ernberg, M. (2015a). Comparison of the Levels of Pro-Inflammatory Cytokines Released in the Vastus Lateralis Muscle of Patients with Fibromyalgia and Healthy Controls during Contractions of the Quadriceps Muscle--A Microdialysis Study. *PLoS One* 10, e0143856.

Christidis, N., Ioannidou, K., Milosevic, M., Segerdahl, M., and Ernberg, M. (2008). Changes of hypertonic saline-induced masseter muscle pain characteristics, by an infusion of the serotonin receptor type 3 antagonist granisetron. *The journal of pain : official journal of the American Pain Society* 9, 892-901.

Christidis, N., Kopp, S., and Ernberg, M. (2005). The effect on mechanical pain threshold over human muscles by oral administration of granisetron and diclofenac-sodium. *Pain* 113, 265-270.

Christidis, N., Nilsson, A., Kopp, S., and Ernberg, M. (2007). Intramuscular injection of granisetron into the masseter muscle increases the pressure pain threshold in healthy participants and patients with localized myalgia. *The Clinical journal of pain* 23, 467-472.

Christidis, N., Omrani, S., Fredriksson, L., Gjelset, M., Louca, S., Hedenberg-Magnusson, B., and Ernberg, M. (2015b). Repeated tender point injections of granisetron alleviate chronic myofascial pain--a randomized, controlled, double-blinded trial. *J Headache Pain* 16, 104.

Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of health and social behavior* 24, 385-396.

Cook, A.D., Pobjoy, J., Sarros, S., Steidl, S., Durr, M., Lacey, D.C., and Hamilton, J.A. (2013). Granulocyte-macrophage colony-stimulating factor is a key mediator in inflammatory and arthritic pain. *Ann Rheum Dis* 72, 265-270.

- Dahlin, A.P., Wetterhall, M., Caldwell, K.D., Larsson, A., Bergquist, J., Hillered, L., and Hjort, K. (2010). Methodological aspects on microdialysis protein sampling and quantification in biological fluids: an in vitro study on human ventricular CSF. *Analytical chemistry* 82, 4376-4385.
- Damas, J.K., Waehre, T., Yndestad, A., Otterdal, K., Hognestad, A., Solum, N.O., Gullestad, L., Froland, S.S., and Aukrust, P. (2003). Interleukin-7-mediated inflammation in unstable angina: possible role of chemokines and platelets. *Circulation* 107, 2670-2676.
- Dao, T.T., and LeResche, L. (2000). Gender differences in pain. *J Orofac Pain* 14, 169-184; discussion 184-195.
- Dawson, A., Ghafouri, B., Gerdle, B., List, T., Svensson, P., and Ernberg, M. (2013). Pain and intramuscular release of algescic substances in the masseter muscle after experimental tooth-clenching exercises in healthy subjects. *J Orofac Pain* 27, 350-360.
- Dawson, A., Ghafouri, B., Gerdle, B., List, T., Svensson, P., and Ernberg, M. (2015). Effects of experimental tooth clenching on pain and intramuscular release of 5-HT and glutamate in patients with myofascial TMD. *Clin J Pain* 31, 740-749.
- Dawson, A., Ljunggren, L., Ernberg, M., Svensson, P., and List, T. (2014). Effect of experimental tooth clenching on the release of beta-endorphin. *Journal of oral & facial pain and headache* 28, 159-164.
- Delgado, P.L. (2004). Common pathways of depression and pain. *J Clin Psychiatry* 65 Suppl 12, 16-19.
- Dina, O.A., Green, P.G., and Levine, J.D. (2008). Role of interleukin-6 in chronic muscle hyperalgesic priming. *Neuroscience* 152, 521-525.
- Dray, A. (1995). Inflammatory mediators of pain. *British journal of anaesthesia* 75, 125-131.
- Drobek, W., Schoenaers, J., and De Laat, A. (2002). Hormone-dependent fluctuations of pressure pain threshold and tactile threshold of the temporalis and masseter muscle. *Journal of oral rehabilitation* 29, 1042-1051.
- Dubin, A.E., and Patapoutian, A. (2010). Nociceptors: the sensors of the pain pathway. *J Clin Invest* 120, 3760-3772.
- Dworkin, S.F., and LeResche, L. (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6, 301-355.
- Ernberg, M. (2009). Serotonin Receptors. In *Peripheral Receptor Targets for Analgesia Novel Approaches to Pain Management*, B.E. Cairns, ed. (New Jersey, United States: Wiley), pp. 243-274.
- Ernberg, M., Castrillon, E.E., Ghafouri, B., Larsson, B., Gerdle, B., List, T., and Svensson, P. (2013). Experimental myalgia induced by repeated infusion of acidic saline into the human masseter muscle does not cause the release of algescic substances. *Eur J Pain* 17, 539-550.
- Ernberg, M., Hedenberg-Magnusson, B., Alstergren, P., and Kopp, S. (1999). The level of serotonin in the superficial masseter muscle in relation to local pain and allodynia. *Life Sci* 65, 313-325.
- Ernberg, M., Lundeberg, T., and Kopp, S. (2000a). Effect of propranolol and granisetron on experimentally induced pain and allodynia/hyperalgesia by intramuscular injection of serotonin into the human masseter muscle. *Pain* 84, 339-346.

Ernberg, M., Lundeberg, T., and Kopp, S. (2000b). Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals. *Pain* 85, 31-39.

Ernberg, M., Lundeberg, T., and Kopp, S. (2003). Effects on muscle pain by intramuscular injection of granisetron in patients with fibromyalgia. *Pain* 101, 275-282.

Essen, B., and Kaijser, L. (1978). Regulation of glycolysis in intermittent exercise in man. *J Physiol* 281, 499-511.

Ettlin, T. (2004). Trigger point injection treatment with the 5-HT₃ receptor antagonist tropisetron in patients with late whiplash-associated disorder. First results of a multiple case study. *Scandinavian Journal of Rheumatology Supplement* 119, 49-50.

Fanning, N.F., Kell, M.R., Shorten, G.D., Kirwan, W.O., Bouchier-Hayes, D., Cotter, T.G., and Redmond, H.P. (1999). Circulating granulocyte macrophage colony-stimulating factor in plasma of patients with the systemic inflammatory response syndrome delays neutrophil apoptosis through inhibition of spontaneous reactive oxygen species generation. *Shock* 11, 167-174.

Farber, L., Stratz, T.H., Bruckle, W., Spath, M., Pongratz, D., Lautenschlager, J., Kotter, I., Zoller, B., Peter, H.H., Neeck, G., *et al.* (2001). Short-term treatment of primary fibromyalgia with the 5-HT₃-receptor antagonist tropisetron. Results of a randomized, double-blind, placebo-controlled multicenter trial in 418 patients. *International journal of clinical pharmacology research* 21, 1-13.

Farella, M., Soneda, K., Vilmann, A., Thomsen, C.E., and Bakke, M. (2010). Jaw muscle soreness after tooth-clenching depends on force level. *J Dent Res* 89, 717-721.

Fillingim, R.B., and Gear, R.W. (2004). Sex differences in opioid analgesia: clinical and experimental findings. *Eur J Pain* 8, 413-425.

Fillingim, R.B., King, C.D., Ribeiro-Dasilva, M.C., Rahim-Williams, B., and Riley, J.L., 3rd (2009). Sex, gender, and pain: a review of recent clinical and experimental findings. *The journal of pain : official journal of the American Pain Society* 10, 447-485.

Fillingim, R.B., Slade, G.D., Diatchenko, L., Dubner, R., Greenspan, J.D., Knott, C., Ohrbach, R., and Maixner, W. (2011). Summary of findings from the OPPERA baseline case-control study: implications and future directions. *J Pain* 12, T102-107.

Flodgren, G.M., Crenshaw, A.G., Alfredson, H., Fahlstrom, M., Hellstrom, F.B., Bronemo, L., and Djupsjobacka, M. (2005). Glutamate and prostaglandin E₂ in the trapezius muscle of female subjects with chronic muscle pain and controls determined by microdialysis. *Eur J Pain* 9, 511-515.

Forsberg, C., and Bjorvell, H. (1993). Swedish population norms for the GHRI, HI and STAI-state. *Qual Life Res* 2, 349-356.

Frey Law, L.A., Sluka, K.A., McMullen, T., Lee, J., Arendt-Nielsen, L., and Graven-Nielsen, T. (2008). Acidic buffer induced muscle pain evokes referred pain and mechanical hyperalgesia in humans. *Pain* 140, 254-264.

Frot, M., Feine, J.S., and Bushnell, M.C. (2004). Sex differences in pain perception and anxiety. A psychophysical study with topical capsaicin. *Pain* 108, 230-236.

Garcia, E., Godoy-Izquierdo, D., Godoy, J.F., Perez, M., and Lopez-Chicheri, I. (2007). Gender differences in pressure pain threshold in a repeated measures assessment. *Psychology, Health & Medicine* 12, 567-579.

Garland, A., Jordan, J.E., Necheles, J., Alger, L.E., Scully, M.M., Miller, R.J., Ray, D.W., White, S.R., and Solway, J. (1995). Hypertonicity, but not hypothermia, elicits substance P release from rat C-fiber neurons in primary culture. *The Journal of clinical investigation* 95, 2359-2366.

Gater, R., Tansella, M., Korten, A., Tiemens, B.G., Mavreas, V.G., and Olatawura, M.O. (1998). Sex differences in the prevalence and detection of depressive and anxiety disorders in general health care settings: report from the World Health Organization Collaborative Study on Psychological Problems in General Health Care. *Arch Gen Psychiatry* 55, 405-413.

Gear, R.W., Miaskowski, C., Gordon, N.C., Paul, S.M., Heller, P.H., and Levine, J.D. (1996). Kappa-opioids produce significantly greater analgesia in women than in men [see comments]. *Nat Med* 2, 1248-1250.

Gerdle, B., Ghafouri, B., Ernberg, M., and Larsson, B. (2014). Chronic musculoskeletal pain: review of mechanisms and biochemical biomarkers as assessed by the microdialysis technique. *Journal of pain research* 7, 313-326.

Gerdle, B., Hilgenfeldt, U., Larsson, B., Kristiansen, J., Sogaard, K., and Rosendal, L. (2008a). Bradykinin and kallidin levels in the trapezius muscle in patients with work-related trapezius myalgia, in patients with whiplash associated pain, and in healthy controls - A microdialysis study of women. *Pain* 139, 578-587.

Gerdle, B., Lemming, D., Kristiansen, J., Larsson, B., Peolsson, M., and Rosendal, L. (2008b). Biochemical alterations in the trapezius muscle of patients with chronic whiplash associated disorders (WAD)--a microdialysis study. *Eur J Pain* 12, 82-93.

Gerdle, B., Soderberg, K., Salvador Puigvert, L., Rosendal, L., and Larsson, B. (2010). Increased interstitial concentrations of pyruvate and lactate in the trapezius muscle of patients with fibromyalgia: a microdialysis study. *Journal of rehabilitation medicine : official journal of the UEMS European Board of Physical and Rehabilitation Medicine* 42, 679-687.

Gerwin, R.D. (2001). Classification, epidemiology, and natural history of myofascial pain syndrome. *Curr Pain Headache Rep* 5, 412-420.

Ghafouri, B., Larsson, B.K., Sjors, A., Leandersson, P., and Gerdle, B.U. (2010). Interstitial concentration of serotonin is increased in myalgic human trapezius muscle during rest, repetitive work and mental stress - an in vivo microdialysis study. *Scand J Clin Lab Invest* 70, 478-486.

Giordano, J., and Schultea, T. (2004). Serotonin 5-HT(3) receptor mediation of pain and anti-nociception: implications for clinical therapeutics. *Pain Physician* 7, 141-147.

Glennon, R.A., and Dukat, M. (1991). Serotonin receptors and their ligands: a lack of selective agents. *Pharmacology, biochemistry, and behavior* 40, 1009-1017.

Graven-Nielsen, T. (2006). Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. *Scand J Rheumatol Suppl* 122, 1-43.

Graven-Nielsen, T., and Arendt-Nielsen, L. (2003). Induction and assessment of muscle pain, referred pain, and muscular hyperalgesia. *Current pain and headache reports* 7, 443-451.

Graven-Nielsen, T., Arendt-Nielsen, L., Svensson, P., and Jensen, T.S. (1996). Experimental muscle pain: a quantitative study of local and referred pain in humans following injection of hypertonic saline. *J Musculoskel Pain* 5, 49-69.

- Graven-Nielsen, T., Jansson, Y., Segerdahl, M., Kristensen, J.D., Mense, S., Arendt-Nielsen, L., and Sollevi, A. (2003). Experimental pain by ischaemic contractions compared with pain by intramuscular infusions of adenosine and hypertonic saline. *Eur J Pain* 7, 93-102.
- Graven-Nielsen, T., Segerdahl, M., Svensson, P., and Arendt-Nielsen, L. (2001). Methods for induction and assessment of pain in humans with clinical and pharmacological examples. In *Methods in pain research*, L. Kruger, ed. (Boca Raton, Florida: CRC Press), pp. 264-304.
- Greenspan, J.D., Slade, G.D., Bair, E., Dubner, R., Fillingim, R.B., Ohrbach, R., Knott, C., Mulkey, F., Rothwell, R., and Maixner, W. (2011). Pain sensitivity risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case control study. *J Pain* 12, T61-74.
- Gur, A., Karakoc, M., Erdogan, S., Nas, K., Cevik, R., and Sarac, A.J. (2002). Regional cerebral blood flow and cytokines in young females with fibromyalgia. *Clin Exp Rheumatol* 20, 753-760.
- Hallberg, L.R., and Carlsson, S.G. (2000). Coping with fibromyalgia. A qualitative study. *Scandinavian journal of caring sciences* 14, 29-36.
- Hammer, C., Cichon, S., Muhleisen, T.W., Haenisch, B., Degenhardt, F., Mattheisen, M., Breuer, R., Witt, S.H., Strohmaier, J., Oruc, L., *et al.* (2012). Replication of functional serotonin receptor type 3A and B variants in bipolar affective disorder: a European multicenter study. *Translational psychiatry* 2, e103.
- Hammer, C., Kapeller, J., Ende, M., Fischer, C., Hebebrand, J., Hinney, A., Friedel, S., Gratacos, M., Estivill, X., Fichter, M., *et al.* (2009). Functional variants of the serotonin receptor type 3A and B gene are associated with eating disorders. *Pharmacogenetics and genomics* 19, 790-799.
- Harada, S., Yamamura, M., Okamoto, H., Morita, Y., Kawashima, M., Aita, T., and Makino, H. (1999). Production of interleukin-7 and interleukin-15 by fibroblast-like synoviocytes from patients with rheumatoid arthritis. *Arthritis Rheum* 42, 1508-1516.
- Headache Classification Committee of the International Headache, S. (2013). The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia* 33, 629-808.
- Hedenberg-Magnusson, B., Brodda Jansen, G., Ernberg, M., and Kopp, S. (2006). Effects of isometric contraction on intramuscular level of neuropeptide Y and local pain perception. *Acta Odontologica Scandinavica* 64, 360-367.
- Helmy, A., Carpenter, K.L., Skepper, J.N., Kirkpatrick, P.J., Pickard, J.D., and Hutchinson, P.J. (2009). Microdialysis of cytokines: methodological considerations, scanning electron microscopy, and determination of relative recovery. *Journal of neurotrauma* 26, 549-561.
- Hillered, L., Valtysson, J., Enblad, P., and Persson, L. (1998). Interstitial glycerol as a marker for membrane phospholipid degradation in the acutely injured human brain. *Journal of neurology, neurosurgery, and psychiatry* 64, 486-491.
- Holland, P.M., Abramson, R.D., Watson, R., and Gelfand, D.H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci U S A* 88, 7276-7280.
- Horjales-Araujo, E., Demontis, D., Lund, E.K., Finnerup, N.B., Borglum, A.D., Jensen, T.S., Svensson, P., and Vase, L. (2013). Polymorphism in serotonin receptor 3B is associated with pain catastrophizing. *PLoS One* 8, e78889.

- Huang, G.J., LeResche, L., Critchlow, C.W., Martin, M.D., and Drangsholt, M.T. (2002). Risk factors for diagnostic subgroups of painful temporomandibular disorders (TMD). *J Dent Res* 81, 284-288.
- Isong, U., Gansky, S.A., and Plesh, O. (2008). Temporomandibular joint and muscle disorder-type pain in U.S. adults: the National Health Interview Survey. *J Orofac Pain* 22, 317-322.
- Jeanjean, A.P., Moussaoui, S.M., Maloteaux, J.M., and Laduron, P.M. (1995). Interleukin-1 beta induces long-term increase of axonally transported opiate receptors and substance P. *Neuroscience* 68, 151-157.
- Jensen, K., and Norup, M. (1992). Experimental pain in human temporal muscle induced by hypertonic saline, potassium and acidity. *Cephalalgia* 12, 101-106.
- Johansson, A.G., Nikamo, P., Schalling, M., and Landen, M. (2012). Polymorphisms in AKR1C4 and HSD3B2 and differences in serum DHEAS and progesterone are associated with paranoid ideation during mania or hypomania in bipolar disorder. *Eur Neuropsychopharmacol* 22, 632-640.
- Kadetoff, D., Lampa, J., Westman, M., Andersson, M., and Kosek, E. (2012). Evidence of central inflammation in fibromyalgia-increased cerebrospinal fluid interleukin-8 levels. *Journal of neuroimmunology* 242, 33-38.
- Kapeller, J., Houghton, L.A., Monnikes, H., Walstab, J., Moller, D., Bonisch, H., Burwinkel, B., Autschbach, F., Funke, B., Lasitschka, F., *et al.* (2008). First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. *Human molecular genetics* 17, 2967-2977.
- Kato, M., Fukuda, T., Wakeno, M., Fukuda, K., Okugawa, G., Ikenaga, Y., Yamashita, M., Takekita, Y., Nobuhara, K., Azuma, J., and Kinoshita, T. (2006). Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. *Neuropsychobiology* 53, 186-195.
- Kelley, P., and Clifford, P. (1997). Coping with chronic pain: assessing narrative approaches. *Social work* 42, 266-277.
- Kidd, B.L., and Urban, L.A. (2001). Mechanisms of inflammatory pain. *Br J Anaesth* 87, 3-11.
- Kilpatrick, L.A., Labus, J.S., Coveleskie, K., Hammer, C., Rappold, G., Tillisch, K., Bueller, J.A., Suyenobu, B., Jarcho, J.M., McRoberts, J.A., *et al.* (2011). The HTR3A polymorphism c. -42C>T is associated with amygdala responsiveness in patients with irritable bowel syndrome. *Gastroenterology* 140, 1943-1951.
- King C, Ribeiro-Dasilva M, Wallet S, Fillingim R. (2016). Altered in vitro production of cytokines in temporomandibular disorder (TMD). Abstract PTH 232 presented at: IASP 2016. International Association for the Study of Pain (IASP): 16th World Congress of Pain; Yokohama, Japan.
- Koch, A., Zacharowski, K., Boehm, O., Stevens, M., Lipfert, P., von Giesen, H.J., Wolf, A., and Freynhagen, R. (2007). Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. *Inflamm Res* 56, 32-37.
- Kosek, E., Altawil, R., Kadetoff, D., Finn, A., Westman, M., Le Maitre, E., Andersson, M., Jensen-Urstad, M., and Lampa, J. (2015). Evidence of different mediators of central

- inflammation in dysfunctional and inflammatory pain--interleukin-8 in fibromyalgia and interleukin-1 beta in rheumatoid arthritis. *Journal of neuroimmunology* 280, 49-55.
- Koutris, M., Lobbezoo, F., Naeije, M., Wang, K., Svensson, P., Arendt-Nielsen, L., and Farina, D. (2009). Effects of intense chewing exercises on the masticatory sensory-motor system. *J Dent Res* 88, 658-662.
- Krzywkowski, K., Davies, P.A., Feinberg-Zadek, P.L., Brauner-Osborne, H., and Jensen, A.A. (2008). High-frequency HTR3B variant associated with major depression dramatically augments the signaling of the human 5-HT3AB receptor. *Proc Natl Acad Sci U S A* 105, 722-727.
- Kuru, S., Inukai, A., Liang, Y., Doyu, M., Takano, A., and Sobue, G. (2000). Tumor necrosis factor-alpha expression in muscles of polymyositis and dermatomyositis. *Acta neuropathologica* 99, 585-588.
- Kuryshv, Y.A., Brown, A.M., Wang, L., Benedict, C.R., and Rampe, D. (2000). Interactions of the 5-hydroxytryptamine 3 antagonist class of antiemetic drugs with human cardiac ion channels. *The Journal of pharmacology and experimental therapeutics* 295, 614-620.
- Larsson, B., Rosendal, L., Kristiansen, J., Sjogaard, G., Sogaard, K., Ghafouri, B., Abdiu, A., Kjaer, M., and Gerdle, B. (2008). Responses of algescic and metabolic substances to 8 h of repetitive manual work in myalgic human trapezius muscle. *Pain* 140, 479-490.
- Le Bars, D., Gozariu, M., and Cadden, S.W. (2001). Animal models of nociception. *Pharmacological reviews* 53, 597-652.
- LeResche, L. (1997). Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists* 8, 291-305.
- LeResche, L., Mancl, L., Sherman, J.J., Gandara, B., and Dworkin, S.F. (2003). Changes in temporomandibular pain and other symptoms across the menstrual cycle. *Pain* 106, 253-261.
- Lesurtel, M., Soll, C., Graf, R., and Clavien, P.A. (2008). Role of serotonin in the hepato-gastroIntestinal tract: an old molecule for new perspectives. *Cellular and molecular life sciences : CMLS* 65, 940-952.
- Levine, F.M., and De Simone, L.L. (1991). The effects of experimenter gender on pain report in male and female subjects. *Pain* 44, 69-72.
- Lin, E., Calvano, S.E., and Lowry, S.F. (2000). Inflammatory cytokines and cell response in surgery. *Surgery* 127, 117-126.
- Lindfors, N., Brodin, E., and Ungerstedt, U. (1987). Microdialysis combined with a sensitive radioimmunoassay. A technique for studying in vivo release of neuropeptides. *JPharmacolMeth* 17, 305-312.
- Lobbezoo, F., and Naeije, M. (2001). Bruxism is mainly regulated centrally, not peripherally. *J Oral Rehabil* 28, 1085-1091.
- Lomas, L.M., and Picker, M.J. (2005). Behavioral assessment of temporal summation in the rat: sensitivity to sex, opioids and modulation by NMDA receptor antagonists. *Psychopharmacology (Berl)* 180, 84-94.
- Lund, J.P., Lavigne, G.J., Dubner, R., and Sessle, B.J. (2001). *Orofacial Pain - From Basic Science to Clinical Management*, Vol First (Illinois: Quintessence Publishing Co, Inc).

- Lund, J.P., Sadeghi, S., Athanassiadis, T., Caram Salas, N., Auclair, F., Thivierge, B., Arsenault, I., Rompre, P., Westberg, K.G., and Kolta, A. (2010). Assessment of the potential role of muscle spindle mechanoreceptor afferents in chronic muscle pain in the rat masseter muscle. *PloS one* 5, e11131.
- Lundberg, I.E. (2000). The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. *Current rheumatology reports* 2, 216-224.
- Mackie, A.M., Epstein, J.B., Wu, J.S., and Stevenson-Moore, P. (2000). Nasopharyngeal carcinoma: the role of the dentist in assessment, early diagnosis and care before and after cancer therapy. *Oral oncology* 36, 397-403.
- McMahon, S.B., Cafferty, W.B., and Marchand, F. (2005). Immune and glial cell factors as pain mediators and modulators. *Exp Neurol* 192, 444-462.
- Melzack, R. (1980). Psychologic aspects of pain. *Research publications - Association for Research in Nervous and Mental Disease* 58, 143-154.
- Mengshoel, A.M., Vollestad, N.K., and Forre, O. (1995). Pain and fatigue induced by exercise in fibromyalgia patients and sedentary healthy subjects. *Clin Exp Rheumatol* 13, 477-482.
- Mense, S. (1993). Nociception from skeletal muscle in relation to clinical muscle pain. *Pain* 54, 241-289.
- Mense, S. (2003). The pathogenesis of muscle pain. *Curr Pain Headache Rep* 7, 419-425.
- Merskey, H., and Bogduk, N. (1994). Classification of Chronic pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms, Vol 2nd ed. (Seattle: IASP Press).
- Miles, M.P., and Clarkson, P.M. (1994). Exercise-induced muscle pain, soreness, and cramps. *The Journal of sports medicine and physical fitness* 34, 203-216.
- Monteiro, A.A., and Kopp, S. (1989). The sufficiency of blood flow in human masseter muscle during endurance of biting in the intercuspal position and on a force transducer. *Proc Finn Dent Soc* 85, 261-272.
- Muller, W., and Stratz, T. (2004). Local treatment of tendinopathies and myofascial pain syndromes with the 5-HT₃ receptor antagonist tropisetron. *Scandinavian journal of rheumatology* Supplement (119), 44-48.
- Niesler, B., Flohr, T., Nothen, M.M., Fischer, C., Rietschel, M., Franzek, E., Albus, M., Propping, P., and Rappold, G.A. (2001). Association between the 5' UTR variant C178T of the serotonin receptor gene HTR3A and bipolar affective disorder. *Pharmacogenetics* 11, 471-475.
- Niesler, B., Kapeller, J., Hammer, C., and Rappold, G. (2008). Serotonin type 3 receptor genes: HTR3A, B, C, D, E. *Pharmacogenomics* 9, 501-504.
- Niesters, M., Dahan, A., Kest, B., Zacny, J., Stijnen, T., Aarts, L., and Sarton, E. (2010). Do sex differences exist in opioid analgesia? A systematic review and meta-analysis of human experimental and clinical studies. *Pain* 151, 61-68.
- Nikamo, P., Cheuk, S., Lysell, J., Enerback, C., Bergh, K., Xu Landen, N., Eidsmo, L., and Stahle, M. (2014). Genetic variants of the IL22 promoter associate to onset of psoriasis before puberty and increased IL-22 production in T cells. *The Journal of investigative dermatology* 134, 1535-1541.

- Nikamo, P., Lysell, J., and Stahle, M. (2015). Association with Genetic Variants in the IL-23 and NF-kappaB Pathways Discriminates between Mild and Severe Psoriasis Skin Disease. *The Journal of investigative dermatology* 135, 1969-1976.
- Nilsson, I.M., List, T., and Drangsholt, M. (2005). Prevalence of temporomandibular pain and subsequent dental treatment in Swedish adolescents. *Journal of orofacial pain* 19, 144-150.
- Nordin, M., and Nordin, S. (2013). Psychometric evaluation and normative data of the Swedish version of the 10-item perceived stress scale. *Scandinavian journal of psychology* 54, 502-507.
- Okayasu, I., Oi, K., and De Laat, A. (2009). The effect of tooth clenching on the sensory and pain perception in the oro-facial region of symptom-free men and women. *Journal of oral rehabilitation* 36, 476-482.
- Opal, S.M., and DePalo, V.A. (2000). Anti-inflammatory cytokines. *Chest* 117, 1162-1172.
- Park, J.W., and Chung, J.W. (2016). Inflammatory Cytokines and Sleep Disturbance in Patients with Temporomandibular Disorders. *Journal of oral & facial pain and headache* 30, 27-33.
- Ptolemy, A.S., and Rifai, N. (2010). What is a biomarker? Research investments and lack of clinical integration necessitate a review of biomarker terminology and validation schema. *Scandinavian journal of clinical and laboratory investigation Supplementum* 242, 6-14.
- Reddy, K.S., Naidu, M.U., Rani, P.U., and Rao, T.R. (2012). Human experimental pain models: A review of standardized methods in drug development. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences* 17, 587-595.
- Ren, K., and Dubner, R. (2010). Interactions between the immune and nervous systems in pain. *Nat Med* 16, 1267-1276.
- Rosendal, L., Blangsted, A.K., Kristiansen, J., Sogaard, K., Langberg, H., Sjogaard, G., and Kjaer, M. (2004a). Interstitial muscle lactate, pyruvate and potassium dynamics in the trapezius muscle during repetitive low-force arm movements, measured with microdialysis. *Acta Physiol Scand* 182, 379-388.
- Rosendal, L., Larsson, B., Kristiansen, J., Peolsson, M., Sogaard, K., Kjaer, M., Sorensen, J., and Gerdle, B. (2004b). Increase in muscle nociceptive substances and anaerobic metabolism in patients with trapezius myalgia: microdialysis in rest and during exercise. *Pain* 112, 324-334.
- Ruano, G., Thompson, P.D., Windemuth, A., Seip, R.L., Dande, A., Sorokin, A., Kocherla, M., Smith, A., Holford, T.R., and Wu, A.H. (2007). Physiogenomic association of statin-related myalgia to serotonin receptors. *Muscle Nerve* 36, 329-335.
- Sarnat, B.G., and Laskin, D.M. (1992). *The temporomandibular joint: A biological basis for clinical practice*, Vol Fourth (Philadelphia: W.B. Saunders Company).
- Schafers, M., Sorkin, L.S., and Sommer, C. (2003). Intramuscular injection of tumor necrosis factor-alpha induces muscle hyperalgesia in rats. *Pain* 104, 579-588.
- Scheller, D., and Kolb, J. (1991). The internal reference technique in microdialysis: a practical approach to monitoring dialysis efficiency and to calculating tissue concentration from dialysate samples. *Journal of neuroscience methods* 40, 31-38.
- Schiffman, E., Ohrbach, R., Truelove, E., Look, J., Anderson, G., Goulet, J.P., List, T., Svensson, P., Gonzalez, Y., Lobbezoo, F., *et al.* (2014). Diagnostic Criteria for

Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Groupdagger. *Journal of oral & facial pain and headache* 28, 6-27.

Schneider, E.M., Ma, X., Stratz, T., Mâ • ¼ller, W., Lorenz, I., and Seeling, W.D. (2004). Immunomodulatory function of the 5-HT₃ receptor antagonist tropisetron. *Scandinavian Journal of Rheumatology Supplement*, 34-40.

Scholz, J., and Woolf, C.J. (2002). Can we conquer pain? *Nature neuroscience* 5 Suppl, 1062-1067.

Schweizer, A., Feige, U., Fontana, A., Muller, K., and Dinarello, C.A. (1988). Interleukin-1 enhances pain reflexes. Mediation through increased prostaglandin E₂ levels. *Agents and actions* 25, 246-251.

Scott, D.S., and Lundeen, T.F. (1980). Myofascial pain involving the masticatory muscles: an experimental model. *Pain* 8, 207-215.

Seide, M.F., Ulrich-Merzenich, G., Fiebich, B., Candelario-Jalil, E., Koch, F.W., and Vetter, H. (2004). Tropisetron inhibits serotonin-induced PGE₂ release from macrophage-like synovial cells in serum-free tissue culture. *Scandinavian journal of rheumatology Supplement* (119), 33.

Sessle, B.J. (1999). The neural basis of temporomandibular joint and masticatory muscle pain. *Journal of orofacial pain* 13, 238-245.

Shah, J.P., Danoff, J.V., Desai, M.J., Parikh, S., Nakamura, L.Y., Phillips, T.M., and Gerber, L.H. (2008). Biochemicals associated with pain and inflammation are elevated in sites near to and remote from active myofascial trigger points. *Archives of Physical Medicine and Rehabilitation* 89, 16-23.

Shah, J.P., Phillips, T.M., Danoff, J.V., and Gerber, L.H. (2005). An in vivo microanalytical technique for measuring the local biochemical milieu of human skeletal muscle. *J Appl Physiol* 99, 1977-1984.

Sherman, J.J., and Leresche, L. (2006). Does Experimental Pain Response Vary across the Menstrual Cycle? A Methodologic Review. *American Journal of PhysiologyRegulatory, Integrative and Comparative Physiology*.

Sjolund, B.H., and Persson, A.L. (2007). Pressure pain threshold changes after repeated mechano-nociceptive stimulation of the trapezius muscle: possible influence of previous pain experience. *J Pain* 8, 355-362.

Slade, G.D., Bair, E., By, K., Mulkey, F., Baraian, C., Rothwell, R., Reynolds, M., Miller, V., Gonzalez, Y., Gordon, S., *et al.* (2011a). Study methods, recruitment, sociodemographic findings, and demographic representativeness in the OPPERA study. *J Pain* 12, T12-26.

Slade, G.D., Conrad, M.S., Diatchenko, L., Rashid, N.U., Zhong, S., Smith, S., Rhodes, J., Medvedev, A., Makarov, S., Maixner, W., and Nackley, A.G. (2011b). Cytokine biomarkers and chronic pain: association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness. *Pain* 152, 2802-2812.

Slade, G.D., Smith, S.B., Zaykin, D.V., Tchivileva, I.E., Gibson, D.G., Yuryev, A., Mazo, I., Bair, E., Fillingim, R., Ohrbach, R., *et al.* (2013). Facial pain with localized and widespread manifestations: separate pathways of vulnerability. *Pain* 154, 2335-2343.

Sluka, K.A., Kalra, A., and Moore, S.A. (2001). Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. *Muscle & nerve* 24, 37-46.

- Smith, S.B., Maixner, D.W., and Greenspan, J.D. (2011). Potential genetic risk factors for chronic TMD: genetic associations from the OPPERA case control study. *J Pain* 12, 92-101.
- Smith, S.B., Mir, E., Bair, E., Slade, G.D., Dubner, R., Fillingim, R.B., Greenspan, J.D., Ohrbach, R., Knott, C., Weir, B., *et al.* (2013). Genetic variants associated with development of TMD and its intermediate phenotypes: the genetic architecture of TMD in the OPPERA prospective cohort study. *J Pain* 14, T91-101 e101-103.
- Sommer, C. (2006). Is serotonin hyperalgesic or analgesic? *Current pain and headache reports* 10, 101-106.
- Sommer, C., and Kress, M. (2004). Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neuroscience letters* 361, 184-187.
- Spielberger, C.D. (1975). The measurement of state and trait anxiety: conceptual and methodological issues. In *Emotions-their parameters and measurement*, L. Levi, ed. (New York: Raven Press), pp. 713-725.
- Staahl, C., and Drewes, A.M. (2004). Experimental human pain models: a review of standardised methods for preclinical testing of analgesics. *Basic Clin Pharmacol Toxicol* 95, 97-111.
- Stahle, L., and Borg, N. (2000). Transport of alovudine (3'-fluorothymidine) into the brain and the cerebrospinal fluid of the rat, studied by microdialysis. *Life Sci* 66, 1805-1816.
- Stauber, W.T. (2004). Factors involved in strain-induced injury in skeletal muscles and outcomes of prolonged exposures. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology* 14, 61-70.
- Stohler, C.S., and Lund, J.P. (1994). Effects of noxious stimulation of the jaw muscles on the sensory experience of volunteer human subjects. In *Biological and psychological aspects of orofacial pain*, C.S. Stohler, and D.S. Carlson, eds. (The University of Michigan Center for Human Growth and Development: Ann Arbor), pp. 55-73.
- Stratz, T., Farber, L., and Muller, W. (2002). Local treatment of tendinopathies: a comparison between tropisetron and depot corticosteroids combined with local anesthetics. *Scandinavian journal of rheumatology* 31, 366-370.
- Stratz, T., Fiebich, B., Haus, U., and Muller, W. (2004). Influence of tropisetron on the serum substance P levels in fibromyalgia patients. *Scandinavian journal of rheumatology* Supplement (119), 41-43.
- Stratz, T., and Muller, W. (2004). Treatment of chronic low back pain with tropisetron. *Scand J Rheumatol Suppl* 119, 76-78.
- Strom, V., Roe, C., and Knardahl, S. (2009). Work-induced pain, trapezius blood flux, and muscle activity in workers with chronic shoulder and neck pain. *Pain* 144, 147-155.
- Suarez, E.C., Lewis, J.G., and Kuhn, C. (2002). The relation of aggression, hostility, and anger to lipopolysaccharide-stimulated tumor necrosis factor (TNF)-alpha by blood monocytes from normal men. *Brain Behav Immun* 16, 675-684.
- Svensson, P., Burgaard, A., and Schlosser, S. (2001a). Fatigue and pain in human jaw muscles during a sustained, low-intensity clenching task. *Arch Oral Biol* 46, 773-777.
- Svensson, P., Cairns, B.E., Wang, K., Hu, J.W., Graven-Nielsen, T., Arendt-Nielsen, L., and Sessle, B.J. (2003). Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. *Pain* 101, 221-227.

- Svensson, P., and Kumar, A. (2016). Assessment of risk factors for oro-facial pain and recent developments in classification: implications for management. *J Oral Rehabil.*
- Svensson, P., List, T., and Hector, G. (2001b). Analysis of stimulus-evoked pain in patients with myofascial temporomandibular pain disorders. *Pain* 92, 399-409.
- Svensson, P., Wang, K., Arendt-Nielsen, L., Cairns, B.E., and Sessle, B.J. (2005). Pain effects of glutamate injections into human jaw or neck muscles. *Journal of orofacial pain* 19, 109-118.
- Tegeder, L., Zimmermann, J., Meller, S.T., and Geisslinger, G. (2002). Release of algescic substances in human experimental muscle pain. *Inflammation research : official journal of the European Histamine Research Society [et al]* 51, 393-402.
- Thomas, S.P. (2000). A phenomenologic study of chronic pain. *Western journal of nursing research* 22, 683-699; discussion 699-705.
- Torgimson-Ojerio, B., Ross, R.L., Dieckmann, N.F., Avery, S., Bennett, R.M., Jones, K.D., Guarino, A.J., and Wood, L.J. (2014). Preliminary evidence of a blunted anti-inflammatory response to exhaustive exercise in fibromyalgia. *Journal of neuroimmunology* 277, 160-167.
- Toritsu, T., Wang, K., Svensson, P., De Laat, A., Fujii, H., and Arendt-Nielsen, L. (2007). Effect of low-level clenching and subsequent muscle pain on exteroceptive suppression and resting muscle activity in human jaw muscles. *Clin Neurophysiol* 118, 999-1009.
- Treede, R.D., Rief, W., Barke, A., Aziz, Q., Bennett, M.I., Benoliel, R., Cohen, M., Evers, S., Finnerup, N.B., First, M.B., *et al.* (2015). A classification of chronic pain for ICD-11. *Pain* 156, 1003-1007.
- Uceyler, N., Valenza, R., Stock, M., Schedel, R., Sprötte, G., and Sommer, C. (2006). Reduced levels of antiinflammatory cytokines in patients with chronic widespread pain. *Arthritis Rheum* 54, 2656-2664.
- Ungerstedt, U. (1991). Microdialysis--principles and applications for studies in animals and man. *Journal of internal medicine* 230, 365-373.
- Wall, P.D., and Melzack, R. (1994). *Textbook of pain, Vol Third* (London: Churchill Livingstone).
- van Hall, G., Sacchetti, M., Radegran, G., and Saltin, B. (2002). Human skeletal muscle fatty acid and glycerol metabolism during rest, exercise and recovery. *J Physiol* 543, 1047-1058.
- Warren, M.P., and Fried, J.L. (2001). Temporomandibular disorders and hormones in women. *Cells Tissues Organs* 169, 187-192.
- Watkins, L.R., and Maier, S.F. (2005). Immune regulation of central nervous system functions: from sickness responses to pathological pain. *Journal of internal medicine* 257, 139-155.
- Vecchiet, L., Dragani, L., Bigontina, P., Obletter, G., and Giamberardino, M.A. (1993). Experimental referred pain and hyperalgesia from muscles in humans. In *New trends in referred pain and hyperalgesia*, L. Vecchiet, D. Albe-Fessard, and U. Lindblom, eds. (Amsterdam: Elsevier), pp. 239-249.
- Velly, A.M., Gornitsky, M., and Philippe, P. (2003). Contributing factors to chronic myofascial pain: a case-control study. *Pain* 104, 491-499.
- Von Korff, M., Dworkin, S.F., and Le Resche, L. (1990). Graded chronic pain status: an epidemiologic evaluation. *Pain* 40, 279-291.

Von Korff, M.R., Howard, J.A., Truelove, E.L., Sommers, E., Wagner, E.H., and Dworkin, S. (1988). Temporomandibular disorders. Variation in clinical practice. *Medical care* 26, 307-314.

Wong, E.H., Clark, R., Leung, E., Loury, D., Bonhaus, D.W., Jakeman, L., Parnes, H., Whiting, R.L., and Eglen, R.M. (1995). The interaction of RS 25259-197, a potent and selective antagonist, with 5-HT₃ receptors, in vitro. *British journal of pharmacology* 114, 851-859.

Voog, O., Alstergren, P., Leibur, E., Kallikorm, R., and Kopp, S. (2000). Immediate effects of the serotonin antagonist granisetron on temporomandibular joint pain in patients with systemic inflammatory disorders. *Life Sciences* 68, 591-602.

Woolf, C.J. (2011). Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 152, S2-15.

Vrabel, M. (2007). Is ondansetron more effective than granisetron for chemotherapy-induced nausea and vomiting? A review of comparative trials. *Clinical journal of oncology nursing* 11, 809-813.

Xing, Z., Gauldie, J., Cox, G., Baumann, H., Jordana, M., Lei, X.F., and Achong, M.K. (1998). IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest* 101, 311-320.

Yamada, K., Hattori, E., Iwayama, Y., Ohnishi, T., Ohba, H., Toyota, T., Takao, H., Minabe, Y., Nakatani, N., Higuchi, T., *et al.* (2006). Distinguishable haplotype blocks in the HTR3A and HTR3B region in the Japanese reveal evidence of association of HTR3B with female major depression. *Biol Psychiatry* 60, 192-201.

Yokoyama, T., Lisi, T.L., Moore, S.A., and Sluka, K.A. (2007). Muscle fatigue increases the probability of developing hyperalgesia in mice. *The journal of pain : official journal of the American Pain Society* 8, 692-699.

Zeitz, K.P., Guy, N., Malmberg, A.B., Dirajlal, S., Martin, W.J., Sun, L., Bonhaus, D.W., Stucky, C.L., Julius, D., and Basbaum, A.I. (2002). The 5-HT₃ subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22, 1010-1019.

Zhang, J.M., and An, J. (2007). Cytokines, inflammation, and pain. *International anesthesiology clinics* 45, 27-37.