Structural Abnormalities of the Temporalis Muscle and Tendon in Temporomandibular Disorders

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science in Oral Radiology

Faculty of Dentistry
University of Toronto

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2019

Abstract

Myalgia of the masticatory muscles is the most common temporomandibular disorder (TMD). Although previous work has explored risk factors, and central and peripheral mechanisms of TMD myalgia (mTMD), the etiology of chronic mTMD pain remains poorly understood.

This project aimed to determine whether chronic mTMD is associated with structural abnormalities of the temporalis muscle. We hypothesized that chronic mTMD is associated with a smaller aponeurosis-tendon complex (ATC) of the temporalis.

T1-weighted magnetic resonance images of 34 chronic mTMD patients and 34 sex- and age matched healthy controls from two cohorts were studied. The volumes of the temporalis muscle and its ATC were measured via software. In both cohorts, mTMD patients had smaller ATC volumes than controls (all $p<0.05$), but similar muscle volumes (all $p>0.05$).

This study demonstrates that chronic mTMD is associated with structural abnormalities of the temporalis.
Acknowledgments

Thank you Drs. Cioffi and Moayedi for your guidance, feedback, and encouragement. Your excitement for this project was infective.

Drs. Davis and Henderson, thank you for allowing me access to your invaluable data. Without you this project would not be possible.

Dr. Agur, thank you for your patience, knowledge, your structure, and your time.

Dr. Lam, thank you for your insight and for giving me perspective. Thank you for allowing us the time to learn, read, and explore.

I would like to acknowledge those individuals who donated their bodies and tissue for the advancement of education and research.

I would like to thank my co-residents, past and present, who have guided me through this journey. Your support and feedback has made me a better student.

Trevor, thank you for being an incredible co-resident. I hope you learned from me half of what I have learned from you. Your positivity and knowledge has been invaluable. Thank you for always being there.

Thank you to my family, without whom none of this would be possible.
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<th>Full Form</th>
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<tr>
<td>AP</td>
<td>Anterior-Posterior</td>
</tr>
<tr>
<td>ATC</td>
<td>Aponeurotic tendon complex</td>
</tr>
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<td>CN V</td>
<td>Cranial nerve five</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CTR</td>
<td>Control</td>
</tr>
<tr>
<td>DT</td>
<td>Deep part of the temporalis</td>
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<tr>
<td>GE</td>
<td>Gradient echo</td>
</tr>
<tr>
<td>LL</td>
<td>Lateral</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging/images</td>
</tr>
<tr>
<td>mTMD</td>
<td>TMD myalgia</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SI</td>
<td>Superior-Inferior</td>
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<tr>
<td>ST</td>
<td>Superficial part of the temporalis</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TMD</td>
<td>Temporomandibular disorder</td>
</tr>
<tr>
<td>TMJ</td>
<td>Temporomandibular joint</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
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<tr>
<td>ZT</td>
<td>Zygomatic part of the temporalis</td>
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Chapter 1

1 Introduction

Dental professionals are increasingly tasked with diagnosing and managing patients with orofacial pain conditions (Neville et al., 2015). Temporomandibular disorders (TMDs) are the leading contributor to non-odontogenic orofacial pain and pose a significant healthcare burden (National Institute of Dental and Craniofacial Research, 2018). It is estimated that 5% to 12% of the general population experience one or more episodes of painful TMD with management costs estimated to exceed $4 billion in the United States (National Institute of Dental and Craniofacial Research, 2018).

TMDs are muscle- (myogenous) and/or joint- (arthrogenous) related conditions (Dworkin & LeResche, 1992). Myogenous TMDs (TMD-myalgia; mTMD) are most prevalent, and while they are primarily seen in middle-aged women, they may affect people of any age and sex. TMDs pose a significant impact on quality of life (Fillingim et al., 2011; Greenspan et al., 2011; Maixner et al., 2011; Manfredini et al., 2011; Neville et al., 2015; Ohrbach et al., 2013). Patients with mTMD report moderate to severe dull pain in the jaws and/or temples, and headaches, which are aggravated by function or parafunction (Schiffman et al., 2014). Other characteristics include frequent parafunctional oral behaviors such as awake bruxism, pain catastrophizing behaviours, and mood disorders (Epker & Gatchel, 2000; Lobbezoo et al., 2018; Michelotti et al., 2010; Reiter et al., 2015; Schiffman et al., 2014). In mTMD, the most frequent painful TMD, a clear cause of pain has yet to be identified (Dworkin, 1994; Dworkin & Massoth, 1994; Manfredini et al., 2011).

Recent evidence suggests that patients with chronic mTMD exhibit gray and white matter abnormalities within their central nervous systems (CNS) and structural abnormalities along the trigeminal nerve (CN V) (Moayedi et al., 2012b; Wilcox et al., 2015). Specifically, mTMD patients had lower white matter integrity at the root-entry zone of the trigeminal nerves, and this abnormality was related to the duration of TMD symptomatology, suggesting that the abnormalities were pain-driven (Moayedi et al., 2012b). CNS and CNV abnormalities were also reported by Wilcox et al. (2015) in a different cohort of chronic mTMD patients. These authors found structural abnormalities within the trigeminal nerve, the spinal trigeminal tract, and the
trigeminothalamic tract (Wilcox et al, 2015). Given the convergent evidence of structural abnormalities along the entire trigeminal nociceptive system in chronic mTMD, these findings suggest that the CNS and CN V undergo structural changes in response to a peripheral nociceptive barrage (Gustin et al, 2011a; Moayedi et al, 2011; Moayedi et al, 2012a; Wilcox et al, 2015; Younger et al, 2010; Youssef et al, 2014). However, a clear source of pain in chronic mTMD has yet to be identified. Recent studies have demonstrated that in chronic mTMD, the masseter, a muscle of mastication which is highly active and often painful in patients, has increased concentrations of pro-inflammatory cytokines and reduced tissue reoxygenation after function (Delcanho et al, 1996; Ferreira et al, 2017; Jounger et al, 2017). These functional abnormalities may induce muscle structural changes, which eventually could contribute to a nociceptive barrage in chronic mTMD.

As a first step in understanding the relationships between the muscles of mastication and abnormalities along CN V and in the CNS, I will examine whether patients with chronic mTMD have abnormal temporalis muscle and aponeurotic-tendon complex (ATC) structure. Specifically, this thesis will examine the temporalis muscle and ATC volumes in patients with chronic mTMD and sex- and age-matched pain-free controls.

Should this study demonstrate structural abnormalities in the temporalis muscles of chronic mTMD patients, it will provide novel mechanistic insights into TMD pathophysiology. It would serve as the first step in studying the contribution and possible abnormalities within other masticatory muscles in mTMD. It may suggest that peripheral abnormalities exist in chronic mTMD along with CNS and CN V abnormalities. Possible links between peripheral and central abnormalities in mTMD can be investigated in future studies. This may potentially help develop specific treatments to assist patients with chronic mTMD. Further, strategies may be developed to prevent disease and reduce the healthcare burden.
Chapter 2

2 Literature review

2.1 Temporomandibular disorders

The International Association for the Study of Pain defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey, 1991; Merskey, 1986). Pain motivates us to protect potentially damaged tissue from further injury. Acute pain is usually defined as occurring under three months while chronic pain is usually defined as persistent or recurrent pain for three or more months, or greater than the normal timeframe for tissue healing (Scholz et al, 2019). It must be recognized that pain is subjective, experiential, and may have a multifactorial etiology.

Temporomandibular disorders are recognized by the National Institute of Dental and Craniofacial Research as a group of musculoskeletal and neuromuscular conditions that involve the temporomandibular joints (TMJs), the masticatory muscles, and all associated tissues (National Institute of Dental and Craniofacial Research, 2018). TMDs are the second most common musculoskeletal condition after lower back pain (National Institute of Dental and Craniofacial Research, 2018). TMD-related pain impacts psychosocial functioning and quality of life significantly (Fillingim et al, 2011; Kafas & Leeson, 2006), posing a significant public healthcare burden. In the United States, painful TMD is estimated to account for an annual loss of over 17.8 million workdays (Wadhwa & Kapila, 2008).

The recent taxonomy (see section 2.3 for classification of TMDs) distinguishes TMDs as non-painful and painful (Peck et al, 2014). For instance, TMJ derangement (e.g. joint clicking) may not be painful (Okeson, 2014). Conversely, TMD myalgia (mTMD) and arthralgia are characterized by dull pain within and around the muscles of mastication and TMJ that is affected by jaw movement, function, or parafunction (Schiffman et al, 2014). The treatment of mTMD, the most common form of TMD, is challenging; more than 30% of patients with mTMD have recurrent pain after 5 years of treatment regardless of the treatment they had received (Rammelsberg et al, 2003). Management strategies for mTMD include medication (e.g. non-steroidal anti-inflammatory drugs, muscle relaxants, and benzodiazepines), psychological
therapy (e.g. cognitive behavioural therapy), occlusal splints, and physiotherapy (Gil-Martínez et al, 2018; Haggman-Henrikson et al, 2017).

Characterization and classification of TMDs (through the Research Diagnostic Criteria for TMD) has improved their diagnoses and treatment. However, the underlying pathophysiology remains poorly understood (Dworkin & LeResche, 1992; Ohrbach & Dworkin, 2016; Schiffman et al, 2014). The current understanding of TMD-pain is based on the biopsychosocial model; inclusive of the biomedical and psychosocial contributors to disease (Suvinen et al, 2005). Purported risk factors for TMD include trauma to the joint or muscles, parafunctional habits, pathophysiologic factors, and psychosocial factors (Carlsson, 1995; Fillingim et al, 2011; Maixner et al, 2011; Ohrbach et al, 2013). The model hypothesizes that a ‘peripheral event’ or injury to the stomatognathic system disrupts normal functioning in a susceptible individual. Examples include motor vehicle accidents (macro-trauma) or more commonly, overload or repetitive strain, injury, and muscle fatigue (micro-trauma) (Suvinen et al, 2005). Inadequate healing may result in prolonged injury and pain.

Many studies have attempted to elucidate mechanisms of TMDs. The initial focus was directed at investigating painful TMJ pathoses (Ahmad et al, 2009; Dworkin & LeResche, 1992; Park et al, 2012; Tasaki & Westesson, 1993; Wiese et al, 2008). Other studies focused on mTMD, the most frequently reported TMD (Manfredini et al, 2011). In the last decade, motivated by quantitative sensory testing studies that showed widespread pain and generalized hyperalgesia — two features of central sensitization — the focus of many research groups shifted to investigating CNS structure and function in chronic mTMD patients (Gustin et al, 2011a; La Touche et al, 2018; Moayedi et al, 2011; Moayedi et al, 2012a; Moayedi et al, 2012b; Sarlani et al, 2004; Wilcox et al, 2015; Younger et al, 2010; Youssef et al, 2014). These studies revealed CNS structural abnormalities in patients with chronic mTMD, along with trigeminal nerve (CNV) abnormalities. These abnormalities suggest a nociceptive barrage from the trigeminal nerve territory in mTMD patients.

There is convergent evidence that the muscles of mastication may be a source of nociceptive drive in mTMD. Specifically, reduced oxygen extraction and abnormal hemodynamics causing reduced muscle re-oxygenation, have been reported in mTMD (Delcanho et al, 1996; Ferreira et al, 2017). These oxygenation abnormalities are thought to be related to tissue injury and
inflammation and may lead to muscular structural changes and increased nociceptive drive. This thesis focuses on studying the structure of a muscle of mastication, the temporalis, in chronic mTMD patients. A review of the trigeminal nerve nociceptive pathway and the stomatognathic system is presented below.

2.2 Anatomy and pathways

2.2.1.1 The temporomandibular joint and masticatory muscles

The United States’ National Library of Medicine defines the stomatognathic (STG) system as relating to the following terms: the mouth, teeth, jaws, pharynx, and related structures as they relate to mastication, deglutition, and speech (United States National Library of Medicine, 2018). The hard tissue structures involve the gnathic bones (maxillae and mandible) and their associated teeth. Posteriorly, the condylar head of the mandible articulates with the complementary glenoid fossa of the temporal bone and facilitates rotational movement, while the articular eminence of the temporal bone allows for translational movement of the mandible. An interarticular disc intervenes between the condylar head and the glenoid fossa to aid in smooth movement. Collectively, this complex series of structures comprise the temporomandibular joint (TMJ). Mandibular movement allows not only for mastication, but for phonation, swallowing, and other related tasks.

![Figure 1: Lateral view of the normal temporomandibular joint anatomy.](image)

An interarticular disc intervenes between the articular eminence and the mandibular condyle (depicted in blue). (Reprinted with permission from Oral Radiology – Principles and Interpretation by White, Pharoah, Mallya, and Lam, 2018).
The mandible is not directly attached to the remainder of the craniofacial osseous structures. Rather, the mandible is suspended from the rest of the cranial bones via three functional ligaments and two accessory ligaments in addition to the muscles of mastication (Okeson, 2014). The three functional ligaments; (1) the collateral ligaments, (2) the capsular ligaments, and (3) the temporomandibular ligaments attach to the mandibular condyle and neck. The bilateral sphenomandibular and stylomandibular ligaments also help limit mandibular movements. These ligaments stretch with prolonged deformation, but do not contribute directly to mandibular movement. Instead, mandibular movements are controlled by four major muscles, collectively termed the muscles of mastication: the lateral and medial pterygoids, the masseter, and the temporalis (Liebgott, 2011).

The lateral pterygoid muscle comprises a superior and inferior head. The superior head arises from the infratemporal surface and inserts into the articular disc and capsule of the TMJ. The inferior head of the lateral pterygoid muscle arises from the lateral surface of the lateral pterygoid plate and inserts onto the mandibular condylar neck. The lateral pterygoid muscle functions to move the mandible anteriorly, inferiorly, and medially. The main belly of the medial pterygoid muscle originates from the medial surface of the lateral pterygoid plate. A smaller portion arises superficially from the maxillary tuberosity. The medial pterygoid muscle attaches along the medial surface of the ramus and the angle of the mandible. The masseter muscle comprises of a superficial and deep head. The superficial head arises from the temporal process of the zygomatic bone and the inferior border of the anterior two-thirds of the zygomatic arch to insert along the lateral surface of the ramus and the angle of the mandible. The deep head of the masseter arises from the inferior border of the posterior one-third and the medial border of the zygomatic arch to insert along the superior half of the lateral surface of the mandibular ramus. Along with the medial pterygoid muscle, the masseter muscle functions to elevate the mandible.

The temporalis muscle arises from the temporal lines along the lateral surface of the skull, the temporal surfaces of the frontal, sphenoid, parietal, and temporal bones, and the zygomatic arch. The temporalis inserts on the coronoid process of the mandible and along the anterior border of the mandibular ramus and functions to elevate and retract the mandible (for further details on the temporalis see section 2.4). Pathology of any component of this complex series of structures is classified as a TMD (Schiffman et al, 2014).
2.2.1.2 Muscle nociception

Nociception refers to the neural process of encoding noxious stimuli (International Association for the Study of Pain, 2019; Merskey, 1991; Merskey, 1986). The specialized class of primary afferent nerve fibers that respond to intense, noxious stimuli are called nociceptors. Thus, pain arising from increased neuronal activity following a noxious or tissue damaging event is termed nociceptive pain. Muscle nociception is thought to be mediated by nociceptive afferents within the walls of arterioles and the connective tissue of muscles (Graven-Nielsen, 2006). Mechanical overloading or trauma can activate muscle nociceptors (Mense, 2008).

Similar to cutaneous nociceptors, molecules such as glutamate, serotonin, nerve growth factor, adenosine triphosphate, hydrogen protons (H\(^+\)), and prostaglandin E\(_2\), among others, are thought to be responsible for endogenous muscle nociception (Cairns et al, 2003; Dong et al, 2006; Graven-Nielsen, 2006; Sessle, 2005). Specifically, nerve growth factor is synthesized in inflamed muscles and activates muscle nociceptors (Mense, 2008). These nociceptors are further activated in acidic environments (low pH) and when muscles generate high adenosine triphosphate (ATP) levels (Mense, 2008). These algesic substances are released during periods of intense muscle activation, upon tissue damage, or under pathologic alterations (Graven-Nielsen, 2006).

Acidic tissue environments, a main contributor to muscle nociception, can be induced in chronic ischemic states, with repeated muscle contractions or spasms (Mense, 2008). In these states, enhanced pain in response to stimuli, termed hyperalgesia, develops at the site of injury (primary hyperalgesia) and in the surrounding uninjured tissue (secondary hyperalgesia) (McMahon et al, 2013). The enhanced responsiveness of nociceptors, called sensitization, accounts for primary hyperalgesia and is due to an increased local release of inflammatory mediators, such as prostaglandin E\(_2\) (Mense, 2008). Secondary hyperalgesia is due to sensitization of neurons within the central nervous system (CNS). As such, patients feel tenderness on muscle palpation and pain upon muscle movement. Muscle nociception differs from cutaneous nociception in that it is poorly localized due to pain referral patterns where pain may be perceived at locations other than the site of the painful stimulus (Nieuwenhuys et al, 2007). This is a result of numerous interconnected sensory neurons that converge from a network of different tissues (as described in section 2.2.1.3). Also, muscle pain is often described as pressing and cramping, whereas
cutaneous pain is often described as burning or stabbing (Mense, 2008). The nociceptive barrage from primary afferent nerve fibers to neurons within the CNS contribute towards hyperalgesia and allodynia.

Primary afferent nerve fibers relay peripherally derived signals to the central nervous system and are classified based on their fiber diameter and myelination, and thus, conduction velocity, as well as the types of receptors expressed in the neurons. While cutaneous primary afferent nerves are classified as Aα, Aβ, Aδ, and C, their muscle counterparts use the Lloyd’s classification system, and the fibers are labelled I – IV, respectively (Mense, 2008). Within muscles, low-threshold mechanoreceptors respond to tactile or stretch stimuli and are responsible for proprioception. These myelinated type Ia and Ib fibers are associated with receptors of the muscle spindle and golgi tendon organ, respectively. Type II (less myelinated) fibers are also associated with receptors of the muscle spindle and together with type I fibers are associated with proprioception. A combination of type II and type III fibers (associated with touch and pressure) are responsible for mechanoception. Type III (thinly myelinated) and IV (non-myelinated) fibers are responsible for the bulk of nociceptive signal conduction (Graven-Nielsen, 2006; Mense, 2008). Nociceptors have free nerve endings – as opposed to other cutaneous fibers which have specialized end organs (Sessle, 2000). The free nerve endings of type IV fibers are unmyelinated axon terminals that directly contact the interstitial fluid of the muscles (Graven-Nielsen, 2006; Mense, 2008). Primary nociceptive afferents from the muscles of mastication project to second order neurons with large receptive fields, thereby contributing to the difficulty in localizing orofacial muscle pain.

2.2.1.3 The trigeminal nociceptive pathways

The muscles of mastication are innervated by the fifth cranial nerve (CN V); the trigeminal nerve. A brief overview of the trigeminal nociceptive pathways is presented (for a comprehensive review, see McMahon et al (2013) and Nieuwenhuys et al (2007); the information reviewed below was primarily derived from these sources).

The trigeminal nerve comprises three branches: the ophthalmic (V1), maxillary (V2), and mandibular (V3) divisions. Each division supplies specified dermatomes and underlying muscular tissue. The trigeminal nerve is largely sensory with minor motor components. These motor components originate in the pons and activate the muscles of mastication along with the
tensor tympani, tensor veli palatini, the anterior belly of the digastric, and the mylohyoid muscles. The sensory afferent innervation is involved in processing converging nociceptive information from the oral, facial, and cranial regions (Mørch et al., 2007; Sessle, 2000). The nociceptive pathways from the periphery to the CNS are distinct for tactile/proprioceptive and nociceptive/thermal stimuli.

Noxious input from the periphery, including the masticatory muscles, is conveyed to the trigeminal ganglion neurons located in the Meckel’s cave. Pertinent to this thesis, the muscles of mastication are innervated by the mandibular (V3) branch with the nerves named according to the muscle they innervate. More specifically, the masseter and temporalis are innervated by the anterior division of V3. Although the trigeminal neurons are somatotopically arranged, the projections from deep tissues, including muscles, exhibit extensive convergence which can complicate pain localization. Also, nociceptive afferents project to various laminae within the dorsal horn of the spinal cord. A practical consequence of these two features of CN V is that individuals may have difficulty localizing and discriminating odontogenic, TMJ, and masticatory muscle pain.

The trigeminal sensory nuclear complex is a relay of sensory information from the craniofacial region to the brain and other regions (see Figure 2). It extends between the midbrain and the upper cervical spinal cord and receives separate input from both nociceptive and non-nociceptive pathways (Bradnam & Barry, 2013; Sessle, 2000). It also receives sensory input from structures outside the distribution of the trigeminal nerve, such as the neck muscles (Sessle, 2000). This may in part explain why mTMD patients often report pain of both the masticatory and neck muscles (Sipilä et al., 2011). The trigeminal sensory nuclear complex comprises the mesencephalic nucleus, the chief sensory nucleus, and the spinal trigeminal nucleus.
Figure 2: The *central connections of the trigeminal nerve*. Position of nerves, tracts and nuclei in a dorsal view. *Roman numerals* indicate the corresponding cranial nerves. V1, Ophthalmic nerve; V2, Maxillary nerve; V3, Mandibular nerve connections of the trigeminal nerve. See text for specifics of the trigeminal nerve nociceptive pathway i.e. 3-5-7-8-9-17-13 from legend (Reprinted with permission from Nieuwenhuys et al, 2007).
The mesencephalic trigeminal nucleus receives afferents associated with proprioception from periodontal receptors and muscle spindles in the muscles of mastication. The chief sensory nucleus receives tactile and discriminative touch inputs from the face, as well as proprioceptive information from the mandible.

The spinal trigeminal nucleus comprises three subregions organized rostro-caudally: oralis, interpolaris, and caudalis. These subnuclei receive nociceptive, mechanical, and thermal inputs from the head and neck including the TMJ, muscles of mastication, and neck muscles (Sessle, 2000). The subnucleus oralis is primarily associated with discriminative tactile information from the orofacial region. The subnucleus interpolaris is also associated with tactile sensation as well as odontogenic nociception. The subnucleus caudalis, located in the medulla, relays tactile, nociceptive, and thermal information (Bradnam & Barry, 2013; McMahon et al, 2013; Sessle, 2000). Masticatory muscle nociceptive afferents primarily terminate within the subnucleus caudalis (Sessle, 2000). It is of note that there are extensive internal projections within the trigeminal sensory nuclear complex, and that neurons of the spinal trigeminal nucleus project caudally to the cervical motor neurons innervating the neck muscles to form reflex loops (Devoize et al, 2010). Interestingly, the subnucleus caudalis, similar to the spinal dorsal horn, has a laminated structure with a similar morphological and functional organization, and is often called the medullary dorsal horn.

Due to the limited work on muscle nociception, we provide an overview combining muscular and cutaneous nociceptive pathways. Within the subnucleus caudalis, the majority of cutaneous nociceptive primary afferents terminate in the marginal zone (lamina I) and substantia gelatinosa (lamina II). The magnocellular layer (laminae III and IV) is equivalent to the nucleus proprius within the dorsal horn and consist of low threshold mecanoceptors and heat, pinch, cold receptors. Wide dynamic range (WDR) neurons are primarily found in laminae V and VI, the deepest zone of the subnucleus caudalis. These deep laminae (V and VI) are the regions of muscle nociceptive primary afferent termination. Cutaneous, odontogenic, meningeal, and muscular input converge within the trigeminothalamic tract thus increasing the difficulty of pain discrimination.

Along with intralaminar projections to lamina II, second-order afferents from the deeper laminae decussate to convey nociceptive information via the trigeminothalamic tract. Unique to CN V,
approximately twenty percent of ascending fibers remain ipsilateral (Roberts & Matzke, 1971; Sessle, 2000). Nociceptive fibers project extensively to the ventroposterior medial and ventroposterior inferior thalamic nuclei, the medial dorsal nucleus, the shell of the ventroposterior complex, and the centrolateral nucleus of the thalamus (Treede et al, 1999). The trigeminal nociceptive input to the thalamus is further relayed to the primary somatosensory cortex, the secondary somatosensory cortex, the insula, and the midcingulate cortex, amongst others brain regions (Treede et al, 1999). This complex pathway is the means by which nociceptive signals are relayed from the muscles of mastication to become a painful perceptual experience created in the CNS. Central nociceptive processing is outside the scope of this thesis, for a comprehensive review, see: (Davis & Moayedi, 2013).

2.3 Classification of TMD

Historical inconsistencies in the TMD diagnostic criteria and classification make comparisons between studies difficult (Manfredini et al, 2011). These inconsistencies are partially due to the lens through which the disease is viewed resulting in different etiologic models. Given that many musculoskeletal (MSK) components of the body function with similar mechanisms, several studies have likened pain arising from the stomatognathic system to other MSK pain disorders (Sipilä et al, 2011; Suvinen et al, 2005). However, it is also contended that the TMJs are unique: the fibrocartilage lined articular surfaces of the TMJ are considered to have a greater capacity for repair compared with hyaline cartilage lined articulations of other joints (Okeson, 2014).

Dworkin and Leresche (1992) proposed a standardized classification system called the Research Diagnostic Criteria for TMD (RDC/TMD), to better understand TMD disease process for research and clinical purposes. The RDC/TMD, based on the biopsychosocial model of pain, was an invaluable first step in facilitating evidence-based research into TMD pathogenesis (Dworkin & LeResche, 1992). The RDC/TMD is a two-axis system: an Axis I clinical assessment accounts for the physical disorder factors and an Axis II assessment accounts for psychosocial status and pain-related disability. Anamnesis and questionnaires help identify putative contributing co-factors for Axis II diagnoses. This organized characterization of the patient’s clinical symptoms and history affords researchers and clinicians improved diagnostic accuracy and patient management.
The RDC/TMD were seen by many in the clinical community as too burdensome, and thus were not widely adopted (Schiffman et al, 2014). Therefore, the Diagnostic Criteria for TMD (DC/TMD) for Clinical and Research Applications was put forth in 2014, which simplified Axis II criteria and increased the specificity and sensitivity of the testing methodologies (Schiffman et al, 2014). These criteria have been widely adopted by both the research and clinical communities and allow for standardized clinical research data acquisition (Ohrbach & Dworkin, 2016). Both criteria split TMDs based on the source of the pathosis and then further categorizes the nature of disease. Accordingly, the DC/TMD criteria are being taught and used in dental schools across North America.

The classification for TMDs within the DC/TMD splits diagnoses into four broad categories; I) TMJ disorders, II) Masticatory Muscle Disorders, III) Headache, and IV) TMD of Associated structures (Schiffman et al, 2014). The full taxonomy of TMD according to the DC/TMD is reported in Figure 3. Of all the listed conditions, disorders of the masticatory muscles (myalgia) are most frequent (Manfredini et al, 2012; Manfredini et al, 2011). Disorders of masticatory muscle myalgia include 1) local myalgia, 2) myofascial pain, and 3) myofascial pain with referral (Schiffman et al, 2014). As noted in section 2.2.1.3, it can be difficult to isolate a diagnosis due to patterns of pain referral and patients may present with overlapping clinical symptoms.
<table>
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<tr>
<th>I. Temporomandibular Joint Disorders</th>
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<tr>
<td>1. Joint pain</td>
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<td>Arthralgia</td>
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<td>Arthritis</td>
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<td>2. Joint Disorders</td>
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<td>A) Disc Disorders</td>
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<td>1. Disc displacement with reduction</td>
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<td>2. Disc displacement with reduction with intermittent locking</td>
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<td>3. Disc displacement without reduction with limited opening</td>
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<td>4. Disc displacement without reduction without limited opening</td>
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<td>B) Hypomobility disorders other than disc disorders</td>
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<td>1. Adhesions/adherence</td>
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<td>2. Arkylosis (Fibrous or Osseous)</td>
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<td>C) Hypermobility disorders</td>
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<td>1. Dislocations</td>
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<td>a) Subluxation b) Luxation</td>
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<td>3. Joint Diseases</td>
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<td>A) Degenerative joint disease</td>
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<td>1. Osteoarthrosis</td>
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<td>2. Osteoarthrits</td>
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<td>B) Systemic arthritides</td>
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<td>C) Condylolysis/idiopathic condylar resorption</td>
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<td>D) Osteochondritis dissecans</td>
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<td>E) Osteonecrosis</td>
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<td>F) Neoplasm</td>
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<td>G) Synovial chondromatosis</td>
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<td>4. Fractures</td>
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<td>5. Congenital/developmental disorders</td>
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<td>A) Aplasia</td>
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<td>B) Hypoplasia</td>
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<td>C) Hyperplasia</td>
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<th>II. Masticatory Muscle Disorders</th>
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<td>1. Muscle Pain</td>
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<td>A) Myalgia</td>
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<td>1. Local myalgia</td>
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<td>2. Myofascial pain</td>
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<td>3. Myofascial pain with referral</td>
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<td>B) Tendonitis</td>
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<td>C) Myotisit</td>
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<td>D) Spasm</td>
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<td>2. Contracture</td>
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<td>3. Hypertrophy</td>
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<td>4. Neoplasm</td>
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<td>5. Movement disorders</td>
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<tr>
<td>A. Orofacial dyskinesia</td>
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<td>B. Oromandibular dystonia</td>
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<td>6. Masticatory muscle pain attributed to systemic/central pain disorders</td>
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<td>Fibromyalgia/widespread pain</td>
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<th>III. Headache</th>
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<td>Headache attributed to TMD</td>
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<th>IV. Associated Structures</th>
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<td>Coronoid hyperplasia</td>
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**Figure 3**: Temporomandibular disorder classification as per the diagnostic criteria of temporomandibular disorders adapted from (Schiffman et al, 2014).
2.3.1.1 TMD-myalgia

TMD myalgia (mTMD) is the most frequent TMD (Manfredini et al, 2011). Unfortunately, chronic mTMD is poorly understood and difficult to manage or treat (Rammelsberg et al, 2003). The term ‘myofascial’ or ‘myalgia’ would suggest that the etiology of pain should arise from the muscle and fascia itself (Benoliel et al, 2011). More accurately, ‘muscle pain’ relates to the conscious perception of pain from the muscle (Graven-Nielsen, 2006). Clinically, this pain can be replicated with palpation of the masticatory muscles, and is affected by jaw movement, function, or parafunction (Schiffman et al, 2014). There is recent evidence for increased inflammation of the masticatory muscles in chronic mTMD (Jounger et al, 2017). Also, there is evidence of reduced post-exercise reoxygenation within these muscles (Delcanho et al, 1996; Ferreira et al, 2017). It is possible that along with these aforementioned functional changes, structural changes within the muscles may exist.

Acute and subacute pain may be related to peripheral nociceptive drive, whereas chronic pain is thought to be maintained by the CNS (Latremoliere & Woolf, 2009; Mense, 2008; Sessle, 2000). Although recent studies have shown CNS and CN V abnormalities in chronic mTMD which help explain generalized hyperalgesia, it is unknown whether peripheral abnormalities exist in mTMD (Moayedi et al, 2011; Moayedi et al, 2012a; Moayedi et al, 2012b; Wilcox et al, 2015; Younger et al, 2010). A first step would be to investigate structural abnormalities within the muscles of mastication in mTMD patients.

2.3.1.2 Epidemiology

TMD myalgias present more frequently in women between the ages of 20 and 40 years old (Levitt & McKinney, 1994; Manfredini et al, 2011; Slade et al, 2011; Tallents et al, 1991). There is evidence to suggest that mTMD is more frequent in those with catastrophizing behavioural traits (Benoliel et al, 2011; Fillingim et al, 2011; Slade et al, 2011). Catastrophizing is characterized by feelings of helplessness, active rumination, and magnification of cognitions towards the painful situation (Quartana et al, 2009). This catastrophic thinking tends to contribute to an increased pain experience as well as increased emotional distress (Sullivan et al, 2001).
In a systematic review on the prevalence of different RDC/TMD Axis I diagnoses in patients and the general population, Manfredini et al. (2011) found a 45.3% prevalence of group I muscle disorder diagnoses. Under the DC/TMD these diagnoses would be classified as II. Masticatory Muscle Disorders. Although prevalence reports varied considerably across studies, myofascial pain with or without mouth opening limitation was the most common diagnosis in TMD patient populations (Manfredini et al, 2012). This corresponds with II.1.A Muscle pain (myalgia) under the DC/TMD. The most consistently painful muscles in mTMD patients are the masseter and the temporalis, the largest jaw closing muscles (Schiffman et al, 2014).

To date, the largest, and most notable TMD epidemiologic data comes from the Orofacial Pain Prospective Evaluation and Risk Assessment Study; the OPPERA Study (Maixner et al, 2011; Slade et al, 2016). This case-control study compared 185 TMD patients with 1,633 TMD-free controls at four study sites in the United States (Slade et al, 2011; Slade et al, 2016). Volunteers (aged 18 to 44) were recruited; 3,263 people without TMD were enrolled into the prospective cohort study; 1,633 of them were selected as controls for the baseline case-control study (Slade et al, 2011). Pertinent to this thesis, sociodemographic comparisons found that women had three times the odds of developing TMDs compared to men, similar to associations observed in the United States population (Slade et al, 2011). Evidence of psychosocial distress, catastrophizing tendencies, and greater pain sensitivity in chronic TMD patients relative to controls were also shown (Fillingim et al, 2011). Although TMD patients have an increased pain experience, given the various factors that influence this subjective measure, studying pain alone poses limits to understating mTMD.

Of interest, oral parafunctional habits, such as tooth clenching which is highly prevalent in mTMD patients, were found to be the strongest risk factor for developing TMD (Cantini et al, 2003; Lobbezoo et al, 2018; Ohrbach et al, 2013). Studies using surface electromyography and ecological momentary assessment have shown that individuals with chronic mTMD have almost four times greater frequency of nonfunctional tooth contacts and tooth clenching episodes as compared to healthy controls (Chen et al, 2007; Cioffi et al, 2017). Therefore, it is plausible that the muscles of mastication of individuals with mTMD undergo structural changes in response to increased non-functional loads.
2.3.1.3 CNS and trigeminal nerve abnormalities in mTMD

Chronic pain could be due to a nociceptive barrage, dysfunctional descending modulation, or a combination of the two. Recently, a series of magnetic resonance imaging (MRI) studies using high-resolution structural brain acquisitions have found gray and white matter abnormalities along the entire ascending nociceptive pathway in mTMD patients (Gustin et al, 2011a; Moayedi et al, 2011; Moayedi et al, 2012a; Moayedi et al, 2012b; Wilcox et al, 2015; Younger et al, 2010). There is also some evidence of volumetric and mean diffusivity changes in descending modulatory regions (Wilcox et al, 2015). Moayedi et al. (2011; 2012a; 2012b) demonstrated that mTMD was associated with structural abnormalities along the entire trigeminal nociceptive pathway, including the trigeminal nerves, the trigeminal brainstem nucleus, the trigeminothalamic tract, the thalamus, thalamocortical tracts to the sensorimotor cortices, and in the primary somatosensory cortex. Similar abnormalities were also reported by Younger et al. (2010) and Wilcox et al. (2015) within the trigeminal nerve, the spinal trigeminal tract, and the trigeminothalamic tract. Notably, mTMD patients had lower white matter integrity at the root-entry zone of the trigeminal nerves, and this abnormality was related to pain duration, suggesting the abnormality may be pain-driven (Moayedi et al, 2012a; Moayedi et al, 2012b). Furthermore, Moayedi (2011; 2012a) also found a correlation between thalamic gray matter and pain duration. Conversely, Gustin et al. (2011a) did not find differences in CNS and trigeminal nerve structure between chronic mTMD patients and healthy controls. However, this may be attributed to a lack of power as a later study by the same group demonstrated structural abnormalities along the trigeminal nociceptive pathway (Wilcox et al, 2015). In sum, these data suggest that there is increased nociceptive drive to the CNS in mTMD, however, the nature of this peripheral barrage is unknown.

Together, the mTMD behavioural data (e.g. parafunctional habits, greater pain sensitivity, psychosocial distress) and the structural CNS and CN V changes suggest a combined barrage of nociceptive peripheral inputs and dysfunctional descending pain modulation. The perception of mTMD is usually different than other orofacial pain conditions: mTMD patients experience dull, lingering pain within the muscles of mastication, exacerbated by palpation (Okeson, 2014; Schiffman et al, 2014). This contrasts with pain experienced by patients with, for example, trigeminal neuralgia, who describe their pain as sharp and stabbing (DeSouza et al, 2014; Gustin et al, 2011b). While trigeminal neuralgia patients’ MRI studies may demonstrate structural injury
or impingement of the trigeminal nociceptive pathway along its course, no such etiology is clear for mTMD (Cairns, 2010; DeSouza et al, 2014; Gustin et al, 2011a; Moayedi et al, 2011; Moayedi et al, 2012b; Schiffman et al, 2014; Youssef et al, 2014). As the source of mTMD pain remains unclear, sources of nociceptive drive, such as the muscles of mastication, should be investigated.

Experimental human models of mTMD have demonstrated increased chemical stimulation of the masticatory muscles may contribute to pain (Cairns et al, 2003; Dong et al, 2006). Algesic chemicals mimic the type of pain reported by mTMD patients, supporting the concept of muscular involvement in mTMD pain (Benoliel et al, 2011; Castrillon et al, 2008). Experimental muscle pain can also be induced by intramuscular electrical stimulation (Graven-Nielsen, 2006; Mense, 2008). Alterations in muscle activity in mTMD patients have been identified by electromyography (EMG), which records the electrical potential of the muscles to attempt to objectively study muscle functional activity (Suvinen & Kemppainen, 2007). If muscles are the source of pain, micro- or macro-structural changes to muscle architecture are likely.

2.3.1.4 Masticatory muscles and the aponeurotic-tendon complex

Skeletal muscle form and function are intrinsically tied, with changes in function modulating musculoskeletal morphology (Russell et al, 2000). The basic unit of the skeletal muscle, the fusiform shaped myocyte, is organized into contractile units known as sarcomeres which work in unison to bring about kinetic change (Liebgott, 2011; Okeson, 2014; Russell et al, 2000). A single muscle can carry out various tasks upon selective activation of motor unit territories based on functional demand (Chang et al, 2013; Eriksson et al, 1984). Often, these territories are delineated by aponeuroses, a variant of the deep fascia, consisting of a sheet of fibrous tissue that attach sheet-like muscles to bone. Pennate muscles, those with oblique muscle fascicles, have aponeuroses that thin into a tendon to attach to bone. Anatomical studies have shown that the masseter and the temporalis, the largest masticatory muscles, present robust aponeurotic layers delineating discrete muscle compartments which represent complex motor unit territories (Chang et al, 2013; Cioffi et al, 2011; Gaudy et al, 2002; Geers et al, 2005). This internal architecture represents an anatomical counterpart of functional heterogeneity and may help explain the large forces produced by these muscles in several spatial dimensions.
In each of the masticatory muscles, muscle compartments and the aponeurotic tendon complexes (ATCs) play a crucial role in functional organization (Chang et al, 2013; Cioffi et al, 2011). For instance, the tendinous architecture of the masseter and its complex internal aponeuroses delineate discrete sub-volumes (muscle compartments), which represent complex motor units (Figure 4) (Ariji et al, 2004; Blanksma et al, 1992; Cioffi et al, 2011).

![Figure 4](image)

**Figure 4: Three-dimensional rendering of the masseter muscle and its aponeuroses.** (a) Cross-sectional scan of a human masseter. (b) Segmentation of muscle boundaries and aponeuroses on consecutive scans. (c) Three-dimensional rendering by software. Note: masseter aponeuroses are identifiable as the low-intensity signal inside the muscle belly. *(Reprinted with permission from Macroscopic analysis of human masseter compartments assessed by magnetic resonance imaging by Cioffi et al, 2011).*

Following the completion of craniofacial growth, there is no further lengthening of skeletal muscles. There may, however, be changes to muscle cross-sectional areas (Russell et al, 2000). Muscles atrophy with disuse and increase in volume to cope with increased functional demand (Russell et al, 2000). Patients with mTMD present with highly frequent oral behaviors (Michelotti et al, 2010; Ohrbach et al, 2013). Theoretically, with the increased load of parafunctional oral habits, the masticatory muscles will be larger in size in the same way that
other skeletal muscles in the body change during strength training programs. However, other features such as reduced reoxygenation and increased inflammation suggest that degenerative changes may result in smaller muscle volumes in mTMD (Ferreira et al, 2017; Jounger et al, 2017). Presently, it is unknown whether the structure of masticatory muscles are different in patients with mTMD. At best, the functional differences noted suggest that mTMD patients may exhibit different muscle volumes compared with control subjects.

Within the muscle fascia, tendons (as part of the ATC) are a highly specialized tissue with a predominately mechanical function (LaCroix et al, 2013). They vary in form, and can be shaped as round cords, ribbons, or strap-like bands (Sharma & Maffulli, 2006). Tenocytes and tenoblasts (immature tenocytes) are the basic cellular elements of the ATC and lie within an extracellular matrix between the collagen fibers of the tendon (Sharma & Maffulli, 2006). ATCs connect muscles and joints, translate muscle contraction into joint movement, and help dissipate the energy produced within the muscles which reduces the likelihood of tissue injury (LaCroix et al, 2013; Thomopoulos et al, 2015). Key functional metrics for pathologic or damaged human tendons include their ultimate strength and elastic modulus (LaCroix et al, 2013). These define the mechanical capabilities of normal tissue and establish risk of failure in disease (LaCroix et al, 2013).

Under normal function, the elastic nature of tendons permit movement by the transmission of forces from muscle fibers to their osseous insertion (Thomopoulos et al, 2015). Though highly adaptive, the ATC exhibits microscopic failure with strain exceeding 4% and macroscopic failure beyond 8-10% strain (Sasaki et al, 1999). Pathology relating to the ATC is termed tendinopathy and is a clinical diagnosis (Kaux et al, 2011; Lui et al, 2011; Sharma & Maffulli, 2006; Thomopoulos et al, 2015). Previously used terms such as tendonitis is now reserved for inflamed tendons studied histologically. In the acutely pathologic state, tendons are thought to respond by undergoing hypertrophy, regeneration, and adaptation to permit anatomic and clinical healing (Cook & Purdam, 2009; Kaux et al, 2011). If unable to adapt to functional demands, or under conditions of excessive load, the ATC exhibit focal areas that lose the normal, aligned, structural organization (Cook & Purdam, 2009; Kaux et al, 2011). Under these conditions, a degenerative process ensues with eventual fragility and loss of ATC architecture (Cook & Purdam, 2009; Kaux et al, 2011). Thus, while acute changes to the ATC may result in edematous and inflammatory changes, chronic dysfunction is likely to result in a reduced ATC volume.
Indeed, increased cytokine levels have been reported within the muscles of mTMD patients and may contribute to tendon degeneration (Jounger et al., 2017).

Hypoxia is a suggested possible cause of tendon degeneration (Cook & Purdam, 2009; Kaux et al., 2011; Sharma & Maffulli, 2006; Thomopoulos et al., 2015). Along with the increased inflammatory milieu, decreased muscle oxygenation is also associated with mTMD muscles (Delcanho et al., 1996). Hypoxia has been shown to promote the expression of inflammatory cytokines and apoptotic mediators that induce tendinopathy (Millar et al., 2012). Such a hypoxic state has been previously demonstrated within the masseter muscle (Delcanho et al., 1996; Ferreira et al., 2017). Thus, it is possible that the hypoxic state of masticatory muscles in chronic mTMD patients may result in inflammation and ATC degenerative changes. Changes in the muscle and ATC structures of the masticatory muscles may be one of the possible peripheral sources of increased nociceptive drive. Therefore, an in vivo analysis of the masticatory muscles and ATC structure in mTMD may reflect an underlying pathologic cause for pain.

Previous studies have suggested that architectural changes can occur in the contractive and connective tissue elements within the masticatory muscles of TMD patients (Ariji et al., 2010; D’Ippolito et al., 2010; Lopes et al., 2015; Taskaya-Yilmaz et al., 2005). However, these studies do not clearly demonstrate structural differences. Ariji et al. (2004) reported reduced masseter muscle thickness and disorganization/destruction of aponeuroses in mTMD patients. However, they measured maximal masseter thickness (using a single location) and subjectively evaluated its internal ATC by its visibility and width, finding thicker masseter muscles in mTMD patients at rest. However, a study by Proveda-Roda and colleagues (2018) did not find differences in masseter structure between mTMD patients and controls. Using magnetic resonance (MR) imaging, Taskaya-Yilmaz et al. (2005), Lopes et al. (2015), and D’ippolito et al. (2010) reported differences in the lateral pterygoid muscle (LPM) of TMD patients. Taskaya-Yilmaz et al. (2005) and D’ippolito et al. (2010) reported that the LPM were atrophied and degenerated in TMD patients based on subjective interpretations. A key shortcoming of these two studies is that they did not evaluate chronic mTMD; Taskaya-Yilmaz et al. (2005) evaluated TMD patients with joint pathoses and D’ippolito et al. (2010) did not report TMD diagnoses.

In contrast, Lopes et al. (2015) reported LPM hypertrophy in patients with TMD and migraines. In this study however, patients with TMD were used as the control group; no pain-free controls
were included. Consequently, much of the evidence for muscular abnormalities from the LPM
should be interpreted with caution. Moreover, the LPM cannot be reliably palpated clinically to
confirm its contribution to mTMD (Schiffman et al, 2014). The masseter and temporalis muscles
are more reliable from a clinical standpoint and are relatively easy to palpate and study as they
reside under the skin surface in accessible areas of the face (Schiffman et al, 2014). Furthermore,
these are most consistently the location of mTMD pain (Schiffman et al, 2014).

Masseter muscle thickness has been studied using ultrasonography with relative ease given a
lack of impeding anatomy. There are large variations in masseter thickness across individuals, as
determined by ultrasonography (Kiliaridis & Kälebo, 1991). Due to the complex architecture of
the masseter, with multiple internal ATC bands, the heterogeneity makes comparisons in a
limited sample size difficult (Cioffi et al, 2011). The variations in masseter thickness and
inconsistencies with ultrasonography studies limits the interpretability of masseter muscle
thickness comparisons alone (Reis Durao et al, 2017). Therefore, studying a muscle with simpler
architecture that is still frequently involved in mTMD for proof of principle is better.

There have been few, if any, studies of the variability of temporalis muscle structure. Clinically,
some TMD patients report tension headaches, that some authors attribute to temporalis
tendon/muscle dysfunction (Chua et al, 1989). Additionally, higher levels of electromyographic
temporalis activity has been reported in mTMD patients (Visser et al, 1995). The large
temporalis muscle with its distinct, single aponeurotic tendon complex (ATC) is an ideal model
for a proof of principle to assess structural abnormalities. A quantifiable measure of the muscle
fibers and the internal ATC is their total volume. Studies in sports medicine have demonstrated
the utility of imaging musculotendinous unit to predict the potential for future injury (Connell et
al, 2004; Fiorentino & Blemker, 2014; Gyftopoulos et al, 2008). In particular, analyses of finite
element models have shown that a decrease in ATC relative to muscle produces higher strains at
the insertion of tendons, which could increase the risk of muscle and tendon injury (Fiorentino &
Blemker, 2014). Therefore, the ATC/muscle volume ratio could be a proxy measure of
dysfunction. As in other muscles, a reduction in the ATC/muscle volume ratio may predict
masticatory muscle injury, which may be associated with chronic nociceptive drive.

It is possible that increased nociceptive drive from an injured temporalis may contribute to
chronic changes within the CNS. Along with other sources, it is possible that the muscle
nociception promotes a barrage of the trigeminal system induced by inflammatory cytokines in an area of tissue damage (Jounger et al., 2017; Mense, 2008). Over time the inflammatory milieu likely results in pathologic tissue damage and structural change. A first step to determining whether the muscles of mastication play a role in mTMD pain would be to assess the volume of a simple masticatory muscle in patients with mTMD, compared to controls.

2.4 Temporalis

The bilateral temporalis muscles play a role in maintaining jaw posture and each have a single, large ATC. mTMD affects the temporalis with reliable clinical diagnostic reproducibility (Schiffman et al., 2014). Further, unlike the masseter muscle, the structure of the temporalis muscle in mTMD has not yet been investigated.

The temporalis is a broad, fan-shaped muscle that occupies the temporal fossa. Most anatomical textbooks describe a simple origin from the temporal lines and fascia in the temporal fossa from the parietal bones and an insertion onto the coronoid process of the mandible (Liebgott, 2011; Sedlmayr et al., 2009). The insertion of the temporalis muscle exists as an ATC. However, anatomical studies specific to the temporalis describe three muscle parts (Figure 5 and 6); a superficial part (ST); a zygomatic part (ZT); and a deep part (DT) (Gaudy et al., 2002; Sedlmayr et al., 2009). As well, extensive interdigitations of the DT with the buccinator and superior pharyngeal constrictor muscles near the retromolar trigone have been described (Sedlmayr et al., 2009).

The superficial temporalis, which corresponds to the temporalis muscle most commonly described in textbooks, originates from the temporal aponeurosis and the temporal lines of the parietal bone, inserting as the tendon onto the coronoid process of the mandible. The anterior edge of the muscle inserts into the temporal surface of the zygomatic process of the frontal bone. The posterior edge of the superficial temporalis inserts into the temporal surface of the temporal process of the zygomatic bone (Gaudy et al., 2002).

The zygomatico-temporalis originates from the medial surface of the zygomatic arch and inserts into the ATC of the superficial temporalis muscle and coronoid process. Antero-inferiorly, the zygomatico-temporalis fuses with the deep masseter (Sedlmayr et al., 2009). Anteriorly, the
zygomatico-temporalis interdigitates with the portion of the deep temporalis originating from the sphenoid bone.

The deep temporalis incorporates the zygomaticomandibularis and the sphenomandibularis that are sometimes described in the literature (Geers et al, 2005; Sedlmayr et al, 2009). It arises from the temporal surfaces of the frontal, sphenoid, parietal, and temporal bones. The muscle fibers extend into the medial surface of the superficial temporalis and the medial aspect of the coronoid process. Also, the deep temporalis that originates from the sphenoid bone inserts into the ATC along the medial coronoid process and anteromedial border of the mandibular ramus as far anteriorly as the retromolar pad.

**Figure 5: Summary of temporalis muscle parts.** Superficial part (a), zygomatic part (b), deep part (c), all parts superimposed (d). All views lateral. Colors: red (superficial part), blue (zygomatic part), green (deep part) (Reprinted with permission from: The human temporalis muscle: Superficial, deep, and zygomatic parts comprise one structural unit by Sedlmayr et al, 2009).
Figure 6: Summary of temporalis muscle part insertions. Superior view of infratemporal fossa (a), inferoposterior view of infratemporal fossa (b), anteromedial view of retromolar triangle and coronoid process (c), anterolateral view of retromolar triangle and coronoid process (d). Colors: red (superficial part), blue (zygomatic part), green (deep part) (Reprinted with permission from: The human temporalis muscle: Superficial, deep, and zygomatic parts comprise one structural unit by Sedlmayr et al, 2009).

The temporalis muscle functions bilaterally during most of its actions. It helps maintain resting tonus, keeping the mandible in its normal upright position. The anterior and middle portions of the muscle elevate the mandible during mastication, approximating the maxillary and mandibular dentition. The posterior fibers lie in a near horizontal position and thus contribute to retrusion of the mandible. During unilateral firing, the temporalis pulls the mandible to the ipsilateral side (Liebgott, 2011). It is not feasible to directly visualize the muscle nor obtain clinical tissue from mTMD patients and controls to investigate structural differences. Thus, imaging remains the gold standard to assess the masticatory muscles in vivo.
Imaging of the masticatory muscles or the TMJ is not always a requirement for TMD diagnosis. It is a test that aids diagnosis or potential therapy. Several imaging modalities have been used to image the TMJ including panoramic imaging, tomography, cone beam computed tomography (CT), and MRI. When patients present with TMD symptoms, a thorough clinical examination and history taking is the first step. If TMJ osseous structure abnormalities are suspected, dentists often prescribe a panoramic image to assess gross changes and may supplement this with cone-beam CTs to further assess the joints. If the clinical examination suggests soft tissues (disc) abnormalities, MR images of the joints are often prescribed. MR offers exquisite soft tissue contrast, and the joint is imaged often in the open and closed mouth positions to observe changes in the position of the interarticular disc. While abnormalities involving the joint have been extensively studied, little is known about the changes and contribution of the muscles of mastication, especially the temporalis, to mTMD.

2.5 Structural assessment of muscles

Muscle architecture is a useful predictor of function as they are fundamentally linked (Russell et al, 2000). Studying muscle architecture can provide a framework for understanding altered muscle function and disease. One measure of muscle and ATC architecture are their volumes. Similar to other muscles, craniofacial skeletal muscle size and cranial dimensions are interdependent, although the exact relationship between the two is unclear (Gionhaku & Lowe, 1989; Hannam & Wood, 1989; Weijs & Hillen, 1986). When studying structural differences in muscles, measures of skull dimensions to determine whether they account for muscle size differences should be considered. In this context, MRI provides both exquisite soft tissue contrast and spatial resolution of craniofacial anatomy.

A brief summary of other imaging modalities that can be used to image the muscles of mastication and their limitations will now be presented for comparison.

Advances in CT technology has allowed for three-dimensional soft tissue and osseous characterization. However, as an extension of conventional radiography, the ionizing radiation required to produce CT images carries an inherent biologic risk. The potential for cancer and heritable effects limits this modality to identifying and assessing neoplastic pathology. More importantly, CT does not allow adequate soft tissue contrast and resolution.
Ultrasonography is a non-invasive modality that has been used to assess the muscles of mastication (Kubo et al, 2006; Reis Durao et al, 2017). The location of the temporalis along the lateral cranial surface lends to ultrasonographic assessment. However, due to the location of the inferior segment of the temporalis muscle and ATC deep the zygomatic arch, anatomy limits ultrasound utilization. The ultrasound waves cannot penetrate the osseous zygomatic arch thus limiting characterization of the inferior segment of the temporalis. Although there has been limited clinical success in treating temporalis tendinopathy using ultrasound guided injections, ultrasonography does not allow for quantitative or qualitative assessment of the entire temporalis (Bressler et al, 2017). MRI, on the other hand, can be used to examine both ATC and muscle structure in humans.

2.6 Magnetic Resonance Imaging

A brief overview on MRI signal and image production and its application to structural muscle imaging will now be presented. For a comprehensive review of the basic concepts of magnetic resonance (MR) and MRI, see Bushberg and Boone (2011) and Dale et al (2015) – the information reviewed below was derived from these sources.

2.6.1.1 Magnetic resonance

Magnetic resonance imaging is based on the spin characteristics of an atomic nucleus in the presence of an applied magnetic field ($B_0$). The nucleus of an atom contains both protons and neutrons, and due to their quantum spin and charge distributions, the nucleus can exhibit magnetic characteristics.

Protons exhibit a positive charge equal to the electron charge but of opposite sign, due to nuclear spin. Neutrons are electrically uncharged but possess nuclear spin of opposite direction but of equal strength to the proton. This sum of spins within a nucleus can be represented as a vector indicating magnitude and direction, termed nuclear magnetic moment or nuclear spin angular momentum. This vector quantity is unique for different elements. Thus, in atoms with even protons and neutrons, the net nuclear magnetic moment is essentially zero. However, if the sum of the protons and neutrons is an uneven number, a net nuclear magnetic moment is generated. Although a single nucleus does not generate a sufficient nuclear magnetic moment to be detected, the combined signal generated from multiple nuclei in a tissue arranged in an
organized, non-random orientation can be detected and analyzed. Due to its abundance within human tissues such as water and fat, hydrogen, with a relatively large magnetic moment, is an ideal atom for generating MR signals.

The spinning proton can be considered as a bar magnet with south and north poles. Due to thermal energy within the body, protons have sufficient energy to spin in random orientations, and thus do not generate a net observable magnetization. However, when placed within a static magnetic field ($B_0$), their magnetic moments align either parallel or anti-parallel to $B_0$. These two orientations, at two discrete energy levels, are the only states in which the protons can exist. Due to opposing $B_0$, the anti-parallel state is at a slightly higher energy level. This results in a slight relative excess of protons in the parallel (lower energy) orientation compared to the anti-parallel (higher energy) state. As the strength of the main magnetic field ($B_0$) increases, the difference between the two energy states increases, and as a result, relatively more protons will reside in the lower energy level. It is this relative excess of protons in the lower energy state that creates an observable nuclear magnetic moment in the same direction as $B_0$. This direction is termed the longitudinal axis ($M_z$).

In addition to separating the protons into two energy states, $B_0$ induces a torque on the protons, causing them to precess around the longitudinal axis. This precession occurs at an angular frequency ($\omega_0$) proportional to the strength of $B_0$. This is described by the Larmour equation and illustrates the dependence between $B_0$ and the angular precessional frequency:

$$\omega_0 = \gamma \times B_0$$

where $\gamma$ is the gyromagnetic ratio which is unique to each element.

This can also be expressed in terms of linear frequency as:

$$f_0 = (\gamma/2\pi) B_0$$

where $\omega = 2\pi f$ and $\gamma/2\pi$ is the gyromagnetic ratio, with values expressed as millions of cycles per second (MHz) per Tesla, or MHz/T.
Each element with a non-zero nuclear magnetic moment has a unique gyromagnetic ratio. In the case of the $^1$H nucleus, $\gamma = 42.58$ MHz/T.

At rest, the net magnetic moment has a vector sum parallel to $B_0$. The plane orthogonal to $M_z$ is called the transverse plane ($M_{xy}$). Selective introduction of a second magnetic field ($B_1$) via a radiofrequency (RF) pulse of varying duration and amplitude changes the equilibrium of the protons within the magnetic field. Protons are promoted from the parallel, low-energy direction to the anti-parallel, higher energy state, shrinking the magnetic vector along the $M_z$ plane. Also, $B_1$ results in phase coherence of the proton spins which produces a net signal in the $M_{xy}$ plane. The protons are now said to be precessing in phase. In short, after the RF pulse, the protons precess in phase around $M_z$ at the Larmour frequency.

2.6.1.2 MR signals

Flip angles represent the degree of rotation of the magnetic moment into $M_{xy}$ by an excitation RF pulse ($B_1$). The RF pulse comprises several frequencies within a narrow range, or bandwidth, which correspond with the protons’ precession rate, as governed by the Larmour equation. The product of the pulse time and the amplitude of $B_1$ determines the displacement of $M_z$. Common flip angles include 90° and 180°. A 90° flip angle provides the largest possible magnetization (signal) in the $M_{xy}$ plane. With flip angles less than 90°, less time is required to displace $M_z$, thus generating a larger transverse magnetization per unit excitation time. For example, a 45° flip angle requires half the time as a 90° flip angle yet generates 70% of the signal. Following $B_1$, there is displacement of the magnetic moment from equilibrium. The absorption of energy of the protons in the magnetized sample, and synchronization of the precessional frequency of the protons generate a rotating vector in the transverse plane $M_{xy}$. This rotating magnetization vector can generate an electrical signal that is detected and forms the basis of the MR signal.

2.6.1.3 Pulse Sequences, T1 and T2 weighting

One of the most commonly used MR images are T1-weighted images. Following a 90° RF pulse applied to a magnetized sample at the Larmour frequency, a maximal $M_{xy}$ is achieved. As the induced RF energy is released back to the surrounding lattice as heat, there is recovery of the net longitudinal magnetization, in a process termed spin-lattice relaxation. Tissues within the human body exhibit different chemical properties (due to the number and chemical composition of
protons) and surrounding environments. Thus, the time taken to return the induced RF energy back to the surrounding lattice (return to equilibrium) is unique to each tissue. This return to equilibrium is also termed T1 relaxation and is the process by which the net magnetization returns to its initial value, parallel to the main magnetic field.

Using spin-echo pulse sequences, T1-weighted images can be acquired by using short repetition (TR: repetition time between successive $B_1$ 90° RF pulses) and short echo times (TE; time after application of the $B_1$ RF excitation pulse and appearance of peak amplitude of the induced echo (when the signal is read)). TE is determined by applying a 180° RF inversion pulse at time TE/2 in order to regain signal amplitude. This is done because following removal of the $B_1$ 90° RF pulse, the protons immediately begin to dephase. Dephasing results in a loss of signal amplitude in the transverse plane. A net signal in the transverse plane is often recorded by the same inducing RF coil (now termed a receive RF coil) and is the basis of the MR image. When the receiver coil is the same as the transmit coil, larger areas of tissue can be imaged simultaneously. However, the use of surface coils to record signals allows for greater spatial resolution, especially when multiple coils are used simultaneously. Note that by using the 180° RF inversion pulse, the time required to regain maximal longitudinal magnetization, and thus the time between successive RF pulses, is lengthened. This makes for lengthy imaging acquisitions. By changing the TR and TE times, T1 or T2 differences in tissues can be emphasized.

T1 is the time constant for regrowth of 63% of the net longitudinal magnetization. In biologic tissues, T1 values range from a few tenths of a second to several seconds. This time depends on proton energy dissipation into the surrounding molecular lattice and hydration layer. The frequencies of the $H^+$ in surrounding lattice and hydration layer can be considered to be tumbling relative to the Larmour precessional frequency of the induced protons. Energy transfer between the protons and the surrounding lattice is most efficient when maximal overlap of these frequencies occurs. The protons within larger, organized molecules such as fat (long carbon chains) efficiently distribute the induced energy to the surrounding lattice. Thus, there is fast recovery of the longitudinal magnetization and fat thus has a short T1 time. Those protons within fluids (smaller molecules) like cerebrospinal fluid (CSF) take longer to recover and thus have a longer T1 time. This is because the energy transfer between the protons and the small, rapidly rotating molecules of free water is a slow process. Muscle has a T1 time that is intermediate,
between that of CSF and fat. These differences in T1 allow for soft tissue contrast in the production of MR images.

Following a 90° RF pulse applied at lower frequency, there is proton phase coherence as well as maximal transverse magnetization. This induces a signal in the receiver antenna coil which produces an electronic signal known as the free-induction decay. This exponential decay process is caused by a loss of phase coherence within the protons due to micro-magnetic inhomogeneities. As the transverse magnetization decays to zero (with concurrent increase in longitudinal magnetization), so does the amplitude and duration of the detected signal. T2 relaxation time describes the time between the peak transfer signal (directly after the $B_1$ 90° RF pulse) and 37% of peak signal. This is mediated by interactions between the magnetic fields of adjacent nuclei spins. T2-weighted images are used to demonstrate tissues with high fluid level but require long TR and TE times.

By acquiring images at a time when the difference in signals from differing tissue (fat/muscle/CSF) is maximized, we are able to produce structural images with excellent soft tissue contrast. The signal recorded from locations within the human body are spatially mapped in three dimensions by the use of magnetic gradients in the three orthogonal planes. A gradient across the main magnetic field corresponding with the axial plane of the patient is termed the slice select gradient. The bandwidth of frequencies excited in the slice select gradient determines the image resolution in the axial plane; the narrower the bandwidth, the more localized the protons excited, the higher the spatial resolution in the axial plane. The ability to induce slightly different phases and frequencies to the protons allows for spatial localization in the remaining x- and y- planes. The gradient that allows for slightly different phase across the imaged sample is known as the phase encoding gradient. The gradient that allows for slightly different frequencies across the imaged sample is known as the frequency encoding gradient. Typically, the smaller length dimension of the region to be imaged is assigned the phase encoding gradient as this allows for more rapid image acquisition. Thus, with the combination of these three gradients, information from the imaged volume can be spatially resolved and assigned discrete voxels (volume elements). The smaller the voxel size, the higher the spatial resolution.

In addition to the T1 and T2 characteristics of tissues, the net level of signal recorded also depends on the number of protons present within the tissue. The higher the number protons, the
larger the net transverse magnetization, thus, the larger the recorded signal. While spin echo pulse sequences produce images with excellent spatial and contrast resolution, they are time intensive due to the time required to regain the net longitudinal magnetization between successive RF pulses. Gradient echo (GE) pulse sequences provide an alternative.

GE technique uses a magnetic field gradient applied in one direction and then reversed to induce formation of an echo instead of the 180° inversion pulse. This allows for purposeful rephasing of the free induction decay. GE imaging uses small flip angles, usually under 60°. The smaller flip angles require less time than those used with spin echo pulse sequences, thus, along with short TRs, allow for much faster image acquisition. Modifications of the GE technique allow for even faster image acquisition. Sequences such as fast spin echo/turbo spin echo use multiple phase encoding gradient steps along with multiple 180° refocusing RF pulses to acquire multiple data acquisitions per TR interval.

As well as localization of the signal, the recorded signal intensities allow for soft tissue contrast. Signals are assigned a pixel grayscale value that reflect the signal intensity. On T1-weighted images, the high signal from fat tissue is displayed as white (bright/high-signal) and the signal from fluids such as the CSF as black (dark/low-signal). Note that regions with low proton concentrations will also appear dark, as will tissues with inefficient spin-lattice energy transfer. Overall, T1-weighted images allow for excellent anatomical assessment of soft tissues.

2.6.1.4 MR Muscle Imaging

Muscle tissue is often of intermediate signal (gray) and can be readily distinguished from adjacent tendon or bone. This is because the adjacent densely calcified tissues such as cortical bone have a low density of protons resulting in minimal T1 signal. Thus, cortical bone appears dark (black). Due to the uniform, linear organization of the collagen and water molecules in healthy tendons, high intrinsic spin-spin (dipole) interactions cause rapid loss of the free induction decay. This results in shortening of the T2 time of tendons to 1-2 ms (Weinreb et al, 2014). Thus, as a result of rapid loss of the transverse magnetization, on T1-weighted images (with short TR and TE times), healthy tendons appear uniformly dark/black. Acquisition of T1-weighted images allows for the anatomic delineation of muscles and tendons. This imaging modality has been validated to study the muscles of mastication (Hannam & Wood, 1989; Lam
et al, 1989; Lam et al, 1991; Schellhas, 1989). Thus, it is the ideal choice for imaging of the temporalis and its ATC.

The force generated by the muscle is inherently tied to its volume. In extra-cranial regions, studies have demonstrated that muscle volumes calculated by comparing muscle cross sectional area on MR images are a major determinant of joint torque (Fukunaga et al, 2001). The muscles are examined orthogonally to the long axis of the muscle fibers and the cross-sectional area is calculated. Often, single anatomic cross-sectional areas are used as a surrogate for total muscle volume because they can accurately estimate total muscle volume (Eng et al, 2007; Morse et al, 2007). MRI can be also be used to determine morphologic changes in specific regions of the muscle with exercise intervention (Hudelmaier et al, 2010). However, these studies investigated the muscles of the limbs and these relationships may not be true for the muscles of mastication (Eng et al, 2007; Morse et al, 2007). These multifunctional muscles have varied movements that are not only involved in mastication but also phonation and swallowing. It is unknown exactly how these muscles adapt to changing functional demands. Further, using a single axial cross-section may not be accurate as there is little data to suggest a representative slice that reflects muscle and ATC volumes. To date, no studies have volumetrically compared the temporalis muscle and ATC between chronic mTMD patients and healthy controls.

2.6.1.5 Anatomical labelling (segmentation)

Segmentation is a method of labelling voxels of a three-dimensional image grid. To characterize differences in muscle and ATC volumes, one needs to essentially count the number of voxels attributed to the tissue of interest. Identified voxels can be counted and multiplied by the voxel dimensions to measure the total volume of a region (tissue) of interest. Open source software such as ITK-SNAP can be used for such purposes (Yasuda et al, 2010; Yushkevich et al, 2006).

Labelling voxels based on anatomic or signal intensity (or both) parameters allows for volume approximation. Also, this segmented area can be reconstructed to examine the structure in three dimensions. This method has previously been used to assess the temporalis muscle volume (Yasuda et al, 2010). Another advantage is the ability to partially automate the segmentation process, thereby reducing human error. When segmenting data, a combination of mapping based on signal intensity and selecting voxels with a device that allows for fine precision is ideal. This ensures maximal inclusion of the tissue of interest and exclusion of adjacent tissue.
To obtain optimal results, the voxel sizes should be small enough to resolve differences between adjacent anatomic structures. Given the narrow lateral dimensions of the temporalis muscle and its ATC, the smallest possible voxel volume is ideal. Larger voxels result in artifacts caused by partial volume averaging and make anatomic delineation less accurate. Studies to assess changes in the brain and spinal cord often use high resolution MR acquisitions with voxel sizes approximating one cubic millimeter (1 mm$^3$) allowing for delineation of minute regions of interest. It is thus feasible to use existing MR data from studies that assessed CNS abnormalities of mTMD patients to assess the temporalis (Moayedi et al, 2012b; Wilcox et al, 2015). The temporalis in mTMD patients and controls can be segmented to obtain the temporalis ATC and muscle volumes and the ATC/muscle volume ratio can be calculated.

### 2.7 Gaps in Knowledge and Rationale

The current data on chronic mTMD does not provide a specific mechanism for pain, but rather identifies several risk factors for the development of mTMD. As outlined in this literature review, one potential contributor to mTMD nociceptive drive are the muscles of mastication and their related structures. To date, there are no consistent and systematic investigations of the muscle, nor ATC structure in chronic mTMD, compared to pain-free controls. Furthermore, there are no \textit{in vivo} studies of structural changes in the musculo-aponeurotic structure of the temporalis muscle in patients with chronic mTMD.

As a first step to determine whether muscle abnormalities contribute to mTMD pain, it is necessary to elucidate musculo-aponeurotic abnormalities in temporalis muscles of patients with chronic mTMD. This may shed novel mechanistic insight and may provide the basis for one potential link between central and peripheral aspects of disease. \textit{No in vivo} studies have investigated temporalis structural differences between chronic mTMD patients and controls. The study of musculo-aponeurotic abnormalities in the temporalis may serve as a first step in elucidating the contributions of masticatory muscles in mTMD and may lay the groundwork for future investigations of masticatory muscles with more complex architecture.
Chapter 3

3 Research Aims and Hypotheses

The objective of this study is to determine whether there are \textit{in vivo} temporalis muscle and ATC volume abnormalities in patients with chronic mTMD, compared to age- and sex-matched pain-free controls.

\textbf{Aim 1:} To delineate the anatomical boundaries of the ATC and the fiber bundles of the temporalis muscle in a cadaveric specimen to inform the \textit{in vivo} data acquisition.

\textbf{Aim 2:} To determine whether patients with chronic mTMD have differing temporalis muscle volumes when compared with control subjects.

Hypothesis: Temporalis muscle volumes differ significantly between patients with chronic mTMD and control subjects.

\textbf{Aim 3:} To determine whether patients with chronic mTMD have differing temporalis ATC/muscle volume ratios compared with control subjects, and to test whether the ATC/muscle volume ratios correlate with pain duration and intensity.

Hypotheses: Temporalis ATC/muscle volume ratio is significantly smaller in patients with chronic mTMD compared with pain-free controls. A smaller temporalis ATC/muscle volume ratio correlates with increased pain duration and intensity in the mTMD cohorts.
Chapter 4

4 Materials and Methods

4.1 Anatomic study

To confirm and standardize anatomic landmarks of the temporalis muscle and ATC during segmentation of the MR images for the purpose of this thesis, an anatomic verification experiment was carried out in cadaver. Landmarks were compared with the images of MR studies.

One hemi-sectioned skull was serially dissected by the principle author of the study (G.K – resident in oral and maxillofacial radiology). First the skin, subcutaneous tissue, and zygomatic arch were removed to expose the underlying temporalis muscle in its entirety. The temporalis fascia was carefully removed to expose the superficial fiber bundles of temporalis. The relationship between the ATC and muscle fibers was examined. Also, the tendon of insertion to the coronoid process was delineated. Fiber bundles were removed individually from the superficial surface of the muscle to expose the entire extent of the ATC within the muscle volume. Osseous landmarks were identified to delineate the extent the ATC within the muscle volume.

4.2 Study of temporalis in patients with chronic mTMD

We identified two existing, independent study cohorts of chronic mTMD patients and age- and sex-matched controls, for whom MR imaging studies had been previously acquired (Moayedi et al, 2012b; Wilcoxon et al, 2015). Both cohorts of patients and controls had been recruited to study CNS abnormalities in mTMD. Collaboration between Professors Luke Henderson (University of Sydney, Australia), and Karen Davis (University of Toronto) allowed access to data collected as part of their projects (Moayedi et al, 2012b; Wilcoxon et al, 2015). These 3D head MR studies that imaged the cranium were confirmed to adequately capture the entirety of the temporalis muscles and ATCs, bilaterally from the skull vertex to the level of the sigmoid notch of the mandible (Moayedi et al, 2012b; Wilcoxon et al, 2015).

Ethics approval was granted by the Health Sciences Research Ethics Board of the University of Toronto (Protocol #: 0036171).
4.2.1 Participants

TMD myalgia patients and controls were voluntarily recruited as part of two independent studies (Moayedi et al, 2012b; Wilcox et al, 2015). The participants recruited at the University of Sydney, Australia will from here on be referred to as the Sydney cohort. The participants recruited at the University of Toronto, Canada will from here on be referred to as the Toronto cohort.

4.2.1.1 Sydney cohort

Patients and controls were voluntarily recruited at the Faculty of Dentistry, Westmead Hospital, University of Sydney (Sydney, New South Wales, Australia). Twenty patients with mTMD were diagnosed using the RDC-TMD (Dworkin & LeResche, 1992). Pain duration (in years) was recorded. Thirty-six healthy controls without facial pain were recruited for the study as this number was required for their study investigating patients with trigeminal neuralgia and TMD. From this dataset, the MRI studies of 17 mTMD patients (mean age ± standard deviation: 42.3 ± 15.7 years, 5 men) were selected based on availability of data from controls. They were age- and sex- matched to a control group of 17 participants (mean age ± standard deviation: 42.9 ± 15.4 years, 5 men).

Patients with mTMD did not have any other known pain conditions. Participants were excluded from the control group if they had any orofacial pain condition or neurologic disorder (Wilcox et al, 2015).

MRI Parameters: The Sydney dataset was acquired using a 3.0 T MRI system (Philips, Intera, Netherlands). Subjects lay supine on the MRI table and their heads were immobilized in a tight-fitting head coil. High-resolution, three-dimensional (3D), T1-weighted anatomical image volumes were acquired, covering the regions of interest (turbo field echo (TFE); echo time: 2.5 milliseconds (ms); repetition time: 5600 ms; flip angle: 8°; voxel dimensions: 0.8 × 0.8 × 0.8 mm³).

4.2.1.2 Toronto cohort

Patients and controls were voluntarily recruited at the Mount Sinai Hospital dental clinic (Toronto, Ontario, Canada). 17 women with mTMD (mean age ± standard deviation: 33.1 ± 11.9
years) were diagnosed using the RDC-TMD (Dworkin & LeResche, 1992). Pain duration (in years) and intensity (numerical score on a 10-point scale) were recorded. As well, 17 age-matched pain-free women (mean age ± standard deviation: 32.8 ± 9.8 years) were recruited. mTMD patients did not have any other known pain disorders. Participants were excluded from the control group if they had a history of chronic pain (Moayed et al, 2012b).

MRI Parameters: The Toronto dataset was acquired using a 3.0 T MRI system (GE Signa HDx, General Electric, USA) with the subject fitted with an eight-channel phased-array head coil. Subjects were placed supine on the MRI table and each subject's head was padded to reduce movement. High-resolution, 3D, T1-weighted anatomical image volumes were acquired, covering the regions of interest (fast spoiled grass sequence with inversion recovery (IR-FSPGR); echo time: 5 ms; repetition time: 12 ms; inversion time: 300 ms; flip angle: 20°; voxel dimensions: 0.94 × 0.94 × 1.5 mm³).

4.3 Segmentation of temporalis muscle and ATC

User-guided segmentation of the temporalis muscles and ATCs (regions of interest, ROI) were carried out using the ITK-SNAP software (Pennsylvania, USA; Yushkevich et al, 2006) on a Huion WH1409 (Shenzhen, China) graphic tablet. All studies were segmented by the principal author of the study (G.K.) who also conducted the anatomic dissection. The operator was blinded to the group assignment of participants from each study cohort.

Tissue contrast was adjusted to optimize distinction of muscle, ATC, and adjacent fat. Each temporalis muscle and ATC were segmented independent of each other and the contralateral side. Using ITK-SNAP’s semiautomatic segmentation pipeline, the T1-weighted MR images were reduced to a single foreground/background thresholding map (blue-white map, Figure 7b) depending on the ROI. Across all studies, ATC signal intensities ranged 50 - 500, and these parameters were used as lower and upper threshold values, respectively. Muscle signal intensities ranged 600 - 1200, and these parameters were used as lower and upper threshold values, respectively. The probability maps of each ROI were refined through comparison to the native image.
Seeds or starting areas of varying sizes were positioned over the ROI where the size reflected efforts to confine the selected voxels to the ROI. This helped prevent spilling of selected voxels into adjacent non-involved spaces with similar signal intensities (e.g. other adjacent muscles). Settings were adjusted to maximize smooth contour boundaries (smoothing (curvature) force = 1.0; region competition force = 1.0). Iterative execution of the ITK-Snap active contour algorithm allowed for selection or contouring of voxels that fulfilled the predetermined parameters for the ROI. The contour expanded, automatically fitting itself to the detailed 3D parameters of the foreground region (Yushkevich et al, 2006). Expansion of the active contour was stopped when voxels outside the ROI were being increasingly selected. This process was repeated for each ROI until the bulk of tissue was contoured. An ad-hoc examination of different planes of segmentation without correction suggests that segmentation of the ROI using only one orthogonal plane may overestimate muscle volume (see Figure 21 in Appendix). Therefore, this automated component of the segmentation was manually refined in all three orthogonal planes by the operator for all MRI data sets. The smooth curve within the polygon toolbar was used to freehand refine the ROI. Each MR data set was segmented in the following order; left ATC, left temporalsmuscle, right ATC, and right temporalsmuscle (Figure 8-10).

![Figure 7: Semi-automatic segmentation of the left temporalis aponeurotic tendon complex (ATC).](image_url)

(a) Native MR image. (b) ATC (white) isolation following thresholding voxel intensities. (c) Seeds positioned over ROI. Execution of the automated iterative ITK-SNAP algorithm. (d) Voxels missed (orange arrow) or incorrectly labelled (yellow arrow) are manually corrected. (e) Final characterization of the left temporalis ATC.
Following the first segmentation, each MR study was verified in all orthogonal planes after a one-week washout. Any disagreement was resolved following discussion with Drs. Agur (expert in anatomy) and Cioffi (expert in MR imaging of the muscles of mastication). The volumetric data was then recorded for data analysis. ITK-Snap calculates the total ROI volume as a product of the number of selected voxels and voxel size. From this data, the temporalis ATC/muscle volume ratio was computed.

Figure 8: Axial T1-weighted MR image with segmentation of the left temporalis muscle (red) and left ATC (green). Right side not segmented for illustrative comparison.
Figure 9: Segmented (a) axial, (b) coronal, and (c) sagittal images of the temporalis. Left ATC (green) and muscle (red). Right ATC (yellow) and muscle (blue). Unsegmented right ATC/muscle is shown in the coronal slice (b) for comparison.

Figure 10: Lateral views of volumetric reconstructions of the a) right and b) left temporalis muscle and ATC. Left ATC (green) and muscle (red). Right ATC (yellow) and muscle (blue).
4.3.1 Recording skull dimensions

To characterize cranial characteristics in the study samples, as an ancillary analysis, the skull dimensions of participants were recorded in the anterior-posterior (AP), superior-inferior (SI), and lateral (LL) planes. This was to assess whether mTMD and control groups had different craniofacial features and was done by counting the number of slices between pre-determined anatomic landmarks. This was converted to a measurement in millimeters by multiplying the number of slices by the voxel dimensions. The landmarks used were: 1) fronto-nasal suture (nasion) to the external occipital protuberance for the anterior-posterior (AP) dimension, 2) skull vertex to superior extent of left zygomatic bone for the superior-inferior (SI) dimension, and 3) between the external acoustic meati for the lateral (LL) dimension.

4.3.2 Intra-rater reliability

To investigate the reproducibility of the segmentation method, within the Sydney cohort, approximately 20% of studies (eight) were reassessed. These were randomly selected; four from the mTMD group and four from the control group. Bilateral segmentation of the temporalis muscle and ATC was conducted two additional times, each one week apart. There was a one-month washout following the initial segmentation for a triplicate of measurements for each ROI.

4.4 Statistical analyses

Descriptive statistics of the temporalis muscle volume, ATC volume, and ATC/muscle volume ratio were obtained across groups.

An intraclass correlation coefficient (ICC) was computed to measure intra-rater reliability, comparing the triplicate measurements of muscle and ATC volumes. An intraclass correlation coefficient less than 0.4, between 0.4 and 0.75, and greater than 0.75 was considered poor, fair to good, and excellent, respectively (Fleiss, 2011).

To test for group differences in muscle and ATC volumes, two multivariate analyses of variance (MANOVA) were used, one for each cohort (Sydney and Toronto). In each MANOVA, temporalis muscle and ATC volumes (logarithmically transformed data) were included as dependent variables, while group (mTMD vs. control), side (left vs. right), and the interaction group*side were included as independent variables.
To test for group differences in the ATC/muscle volume ratio, two univariate analyses of variance were used, one for each cohort (Sydney and Toronto). The ATC/muscle volume ratio was included as the dependent variable, while group (mTMD vs. control), side (left vs. right), and the interaction group*side were included as independent variables. The Bonferroni method was used to adjust for multiple comparisons. Pearson’s correlation coefficients were computed to test correlations between mTMD pain duration and intensity and mean temporalis ATC/muscle volume ratios. To test differences in skull dimensions, unpaired t-tests and Mann-Whitney U tests were used. A difference was considered significant if p <0.05. The statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.
Chapter 5

5 Results

5.1 Anatomic study

The cadaveric dissection of the temporalis via a lateral approach revealed the wide anterior-posterior and superior-inferior extent of the temporalis muscle and its ATC (Figure 11). The superficial temporalis was located deep to the temporalis fascia and attached to the coronoid process of the mandible through the temporalis tendon along its lateral aspect (Figure 12). The short muscle fibers of the zygomatico-temporalis inserted on the superolateral surface of the coronoid process with tendon fibers joining those from the superficial temporalis (Figure 13).

Following removal of the superficial temporalis and zygomatico-temporalis, the full extent of the temporalis ATC could be appreciated (Figure 14). The muscle fibers of the deep temporalis originated from the surface of the infratemporal fossa to insert via the ATC along the medial and anterior borders of the mandibular coronoid process and ramus. Along the ramus, the ATC was tightly adhered to the mandible. Superiorly, above the level of the superior orbital rim, the fibers of the superficial temporalis, ATC, and deep temporalis were indistinguishable.

Similar to prior studies, we were able to also delineate the ATC as the fleshy tendinous portion of the temporalis that inserted around the coronoid process, the anterior portion of the sigmoid notch, and the anterior border of the ramus, extending to the area of the retromolar pad (Geers et al, 2005; Sedlmayr et al, 2009). As suggested by Geers (2005), it was difficult to isolate fibers of the deep temporalis as they were intertwined with fibers of the superficial temporalis.
Figure 11: Lateral view of the temporalis with the superficial skin and fascia removed. The anterior posterior extent of the temporalis is depicted in blue for illustration purposes.

Figure 12: Lateral view of the superficial temporalis (ST) and temporalis tendon (TT) following removal of the zygomatic arch. The temporalis tendon inserts on the coronoid process (Co). The mandibular condyle (C) is shown for reference.
Figure 13: Supero-anterior view of the zygomatico-temporalis (ZT), ATC (TT), and superficial temporalis (ST). Zygomatic arch (ZA) shown for reference.

Figure 14: Images of cadaver with sagittal MRI slices delineating the temporalis muscle and tendon. Muscle (ST): intermediate signal intensity (gray) voxels. Tendon (TT): low signal intensity (black) voxels. Note that the cadaveric and magnetic resonance images are from different subjects.
The cadaveric dissection informed and guided our delineation of the ATC on the MRI studies (Figure 14). Though landmarks on the MR image correspond closely with the cadaveric specimen, exact correlation is not possible as MR images were not of the cadaver.

The superior extent to determine ATC volume was set at the level of the superior orbital rim. Superior to this landmark, the ATC and muscle fibers were indistinguishable in cadaver (Figures 15 and 16). Due to similar T1 tissue characteristics (on MRI), ATC and cortical bone are indistinguishable as both structures appear as regions of low T1 signal intensity. As a result, the inferior extent of the temporalis ATC was determined to be the first axial MRI slice on which the higher signal cancellous bone within the coronoid process of the mandible was visualized. Definition of adjacent muscles via fascia and fat spaces made delineation of the anterior, posterior, medial, and lateral extents of the regions of interest more clearly defined.

**Figure 15: Defined extents of the temporalis ATC.** Superficially the ATC extends to the superior orbital rim. Inferiorly the ATC is defined at the level at which cancellous bone is visualized. Note, the cadaver (lateral view, zygomatic arch removed) and MRI images (sagittal views) are different subjects.
Figure 16: Lateral view of Temporalis depicting extent of the aponeurotic tendon complex (ATC). The white line represents the level of the superior orbital rim, superior to which the fibers of the ATC and muscle are indistinguishable. (A) anterior and (S) superior skull surfaces.

5.2 Comparisons of temporalis muscle and ATC volumes

Descriptive statistics for the left and right temporalis muscles and ATC volumes are reported in Table 1.

The MANOVA revealed that there was no effect of the study group (mTMD vs controls) on the temporalis muscle volumes in both the Sydney and Toronto cohorts (Figure 17, Table 2, and Table 3, Sydney: $F[2, 63] = 1.652, p = 0.203$; Wilk’s $\Lambda = 0.837$, partial $\eta^2 = 0.025$; Toronto: $F[2, 63] = 0.092, p = 0.763$; Wilk’s $\Lambda = 0.671$, partial $\eta^2 = 0.001$).
**Table 1: Descriptive statistics of left and right temporalis (a) muscle volumes (in mm$^3$) and (b) ATC volumes (in mm$^3$) within the Sydney and Toronto cohorts.** Mean and standard deviation (SD). Median and interquartile range [IQR]. * indicates non-normally distributed data. The data from the Sydney and Toronto cohorts were analyzed separately.

(a)

<table>
<thead>
<tr>
<th></th>
<th>Temporals muscle volume (mm$^3$)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean (SD)</td>
<td>median [IQR]</td>
<td>mean (SD)</td>
</tr>
<tr>
<td><strong>Sydney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>34434.69 (6900.34)</td>
<td>32256.05 [11512.93]</td>
<td>32498.30 (6784.05)</td>
</tr>
<tr>
<td>*mTMD</td>
<td>30712.68 (8118.21)</td>
<td>26467.80 [11030.80]</td>
<td>32211.76 (8452.24)</td>
</tr>
<tr>
<td><strong>Toronto</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>30998.59 (5791.31)</td>
<td>32084.80 [11331.45]</td>
<td>32080.75 (7448.17)</td>
</tr>
<tr>
<td>*mTMD</td>
<td>30830.47 (7609.26)</td>
<td>30637.40 [13610.10]</td>
<td>31555.43 (7811.45)</td>
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</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th></th>
<th>Temporals ATC volume (mm$^3$)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean (SD)</td>
<td>median [IQR]</td>
<td>mean (SD)</td>
</tr>
<tr>
<td><strong>Sydney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1977.43 (478.96)</td>
<td>2149.09 [779.67]</td>
<td>2207.54 (504.37)</td>
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<tr>
<td>mTMD</td>
<td>1551.23 (539.43)</td>
<td>1477.68 [950.25]</td>
<td>1715.81 (736.75)</td>
</tr>
<tr>
<td><strong>Toronto</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Controls</td>
<td>3898.47 (967.37)</td>
<td>3564.85 [1493.00]</td>
<td>3922.35 (1041.66)</td>
</tr>
<tr>
<td>*mTMD</td>
<td>2924.51 (688.74)</td>
<td>2830.51 [769.26]</td>
<td>2693.18 (738.91)</td>
</tr>
</tbody>
</table>
Figure 17: Temporalis muscle volumes of control (CTR) and mTMD groups within the Sydney (blue) and Toronto (orange) cohorts. Similar muscle volumes were demonstrated between groups across both independent cohorts (both p>0.05). The data from the Sydney and Toronto cohorts were analyzed separately.

Table 2: Descriptive statistics of the temporalis muscle (in mm$^3$) and ATC volumes (in mm$^3$) of the control and mTMD groups in the Sydney and Toronto cohorts. Mean and standard deviation (SD). Median and interquartile range [IQR]. * indicates non-normally distributed data. The data from the Sydney and Toronto cohorts were analyzed separately.
The MANOVA revealed that the temporalis ATC volumes were smaller in the mTMD group compared with the control group in both the Sydney and Toronto cohorts (all \( p < 0.05 \); Figure 18, Table 2, and Table 3, Sydney: \( F[2, 63] = 12.368, p=0.001; \) Wilk’s \( \Lambda = 0.837, \) partial \( \eta^2 = 0.162 \); Toronto: \( F[2, 63] = 29.601, p < 0.001; \) Wilk’s \( \Lambda = 0.671, \) partial \( \eta^2 = 0.316 \)).

**Figure 18:** Temporalis ATC volumes of control (CTR) and mTMD groups within the Sydney (blue) and Toronto (orange) cohorts. ATC volumes were smaller in the mTMD group compared with the control group across both independent cohorts (\(*\ast\ast\)both \( p < 0.005 \)). The data from the Sydney and Toronto cohorts were analyzed separately.
Table 3: Multivariate analysis of variance (MANOVA). These data provide evidence for significant differences by group (mTMD vs controls), side (left vs right), and a group by side interaction. Logarithmically transformed data (temporalis muscle and ATC volumes) from the Sydney and Toronto cohorts were analyzed separately. df = degrees of freedom. Sig. = significance. Bold type: statistically significant at p<0.05.

**Sydney**

<table>
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<tr>
<th>MANOVA</th>
<th>Dependent variable</th>
<th>df</th>
<th>Wilk's λ</th>
<th>F</th>
<th>Sig.</th>
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<tr>
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<td>0.000</td>
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<td>ATC</td>
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<td>Group</td>
<td>muscle</td>
<td>2, 63</td>
<td>0.837</td>
<td>1.652</td>
<td>0.203</td>
</tr>
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<td>2, 63</td>
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<td>Side</td>
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<td>2, 63</td>
<td>0.974</td>
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<tr>
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<td>0.980</td>
<td>0.815</td>
<td>0.370</td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>2, 63</td>
<td>0.060</td>
<td>0.807</td>
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**Toronto**

<table>
<thead>
<tr>
<th>MANOVA</th>
<th>Dependent variable</th>
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<th>Wilk's λ</th>
<th>F</th>
<th>Sig.</th>
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<tr>
<td></td>
<td>ATC</td>
<td>2, 63</td>
<td>10.245</td>
<td>&lt;0.001</td>
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<tr>
<td>Group</td>
<td>muscle</td>
<td>2, 63</td>
<td>0.671</td>
<td>0.092</td>
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</tr>
<tr>
<td></td>
<td>ATC</td>
<td>2, 63</td>
<td>29.601</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td>Side</td>
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<td>0.177</td>
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<td>0.531</td>
<td>0.469</td>
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<tr>
<td>Group * Side</td>
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<td>0.979</td>
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<tr>
<td></td>
<td>ATC</td>
<td>2, 63</td>
<td>0.602</td>
<td>0.441</td>
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mTMD patients had a smaller temporalis ATC/muscle volume ratio compared with controls in both the Sydney and Toronto cohorts (Sydney p = 0.010, Toronto p < 0.001; Figure 19 and Table 5). Descriptive statistics for the temporalis ATC/muscle volume ratio is reported in Table 4. Note that the data from the Sydney and Toronto cohorts were analyzed separately.

![Box plots showing ATC/muscle volume ratio comparison between CTR and mTMD in Sydney and Toronto cohorts](image)

**Figure 19:** Temporalis ATC/muscle ratio of mTMD patients and controls within the Sydney (blue) and Toronto (orange) cohorts (*p<0.05; **p<0.005). The data from the Sydney and Toronto cohorts were analyzed separately.
Table 4: Descriptive statistics of the temporalis ATC/muscle volume ratio of control and mTMD groups in the Sydney and Toronto cohorts. Mean and standard deviation (SD). Median and interquartile range [IQR]. * indicates non-normally distributed data. The data from the Sydney and Toronto cohorts were analyzed separately.

<table>
<thead>
<tr>
<th>Temporalis ATC/Muscle volume ratio (%)</th>
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<th>median [IQR]</th>
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<tr>
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<td>6.40 (1.74)</td>
<td>5.91 [3.21]</td>
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<tr>
<td>mTMD</td>
<td>5.28 (1.77)</td>
<td>5.29 [2.04]</td>
</tr>
<tr>
<td><strong>Toronto</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Controls</td>
<td>12.91 (3.95)</td>
<td>13.12 [4.01]</td>
</tr>
<tr>
<td>*mTMD</td>
<td>9.28 (2.41)</td>
<td>9.09 [3.30]</td>
</tr>
</tbody>
</table>

Table 5: Univariate analysis of variance (ANOVA) of the temporalis ATC/muscle volume ratio. These data provide evidence for significant differences by group (mTMD vs controls), side (left vs right), and a group by side interaction. Logarithmically transformed data from the Sydney and Toronto cohorts were analyzed separately. df = degrees of freedom. Sig. = significance. Bold type: statistically significant at p<0.05.

**Sydney**

<table>
<thead>
<tr>
<th>Dependent variable: ratio</th>
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<th>F</th>
<th>Sig.</th>
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<tbody>
<tr>
<td>Corrected model</td>
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<td>3.338</td>
<td>0.025</td>
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<tr>
<td>Group</td>
<td>1</td>
<td>6.981</td>
<td>0.010</td>
</tr>
<tr>
<td>Side</td>
<td>1</td>
<td>1.793</td>
<td>0.185</td>
</tr>
<tr>
<td>Group * Side</td>
<td>1</td>
<td>1.240</td>
<td>0.270</td>
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</tbody>
</table>

**Toronto**

<table>
<thead>
<tr>
<th>Dependent variable: ratio</th>
<th>df</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
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<td>7.182</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group</td>
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<td>20.167</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Side</td>
<td>1</td>
<td>0.950</td>
<td>0.333</td>
</tr>
<tr>
<td>Group * Side</td>
<td>1</td>
<td>0.001</td>
<td>0.516</td>
</tr>
</tbody>
</table>
In both cohorts, our results demonstrated no correlation between the pain duration of mTMD patients and the temporalis ATC/muscle volume ratio (Sydney: \( r = 0.07, p=0.79, \text{with an } R^2 = 0.005 \); Toronto \( r = -0.14, p=0.60, \text{with an } R^2 = 0.019 \)). In the Toronto cohort, there was no correlation between TMD pain intensity of mTMD patients and the temporalis ATC/muscle volume ratio (\( r = 0.24, p=0.35, \text{with an } R^2 = 0.058 \)). A correlation between temporalis ATC/muscle volume ratio and TMD pain intensity from the Sydney cohort was not included due to missing data.

### 5.2.1 Skull measurements

In the Sydney cohort, mTMD patients exhibited larger superior-inferior skull measurements compared with controls (\( p=0.001 \)). However, there was no difference between groups in anterior-posterior and lateral skull measurements (both \( p>0.05 \); Table 6). In the Toronto cohort, mTMD patients exhibited larger antero-posterior skull dimensions (\( p=0.006 \)). However, the superior-inferior and lateral skull measurements were similar between groups (both \( p>0.05 \); Table 6).

#### Table 6: Skull measurements (mm) in the Sydney and Toronto cohorts of mTMD and control patients and between group comparisons. Means and standard deviation (SD) of anterior-posterior (between the fronto-nasal suture and the external occipital protuberance), superior-inferior (between the vertex and the first axial slice of the left zygomatic bone), and lateral (between the external acoustic meati) skull dimensions. See Figure 21. Bold type: statistically significant at \( p<0.05 \).

<table>
<thead>
<tr>
<th></th>
<th>Antero-Posterior</th>
<th>Superior-Inferior</th>
<th>Lateral-Lateral</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>( p )</td>
</tr>
<tr>
<td>Sydney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>160.42</td>
<td>9.38</td>
<td>0.061</td>
</tr>
<tr>
<td>mTMD</td>
<td>166.26</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>Toronto</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>185.51</td>
<td>5.48</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>mTMD</td>
<td>190.70</td>
<td>4.72</td>
<td></td>
</tr>
</tbody>
</table>
Figure 20: Skull measurements (mm) of the mTMD and control groups in the Sydney (blue) and Toronto (orange) cohorts. AP: Anterior-Posterior dimension between the fronto-nasal suture and the external occipital protuberance; SI: Superior-inferior dimension between the vertex and the first axial slice of the left zygomatic bone; LL: Lateral dimension between the external acoustic meati. In the Sydney cohort, mTMD patients demonstrated larger superior-inferior mean skull dimensions relative to controls (***p<0.005). In the Toronto cohort, mTMD patients demonstrated larger anterior-posterior mean skull dimensions relative to controls (*p<0.05).

5.2.2 Intra-rater reliability

In the eight randomly selected subjects that were bilaterally segmented in triplicates following a one-month washout period (after initial segmentation), the intra-rater consistency/reliability was excellent for both the temporalis muscle and the ATC (muscle: Cronbach’s alpha = 0.992; ATC: Cronbach’s alpha = 0.963; Table 7 in Appendix).
Chapter 6

6 Discussion

This thesis aimed to determine whether there are structural masticatory muscle abnormalities in patients with chronic mTMD compared to age- and sex-matched healthy controls. Specifically, we investigated volumetric differences in the total temporalis muscle and its ATC. Given that there are no previous systematic investigations of masticatory muscle structure in mTMD, we investigated the temporalis muscle because of its relatively simple internal architecture, with a single and large ATC. Also, mTMD patients often report pain in this muscle, and it is frequently positive to palpation during the clinical exam (Ohrbach & Dworkin, 2016; Schiffman et al, 2014). Our dissection of the temporalis cadaveric specimen informed the segmentation of the MRI data. We hypothesized that patients with chronic mTMD would exhibit different muscle volumes. However, our results suggest that muscle volumes are not different between mTMD and control groups. We hypothesized that patients with chronic mTMD would exhibit a smaller ATC/muscle volume ratio as compared to healthy controls. Our results supported our hypothesis: we found that chronic mTMD is associated with a reduced ATC volume and a smaller ATC/muscle volume ratio. Notably, the results were reproduced in two independent cohorts of chronic mTMD patients. We hypothesized that a smaller ATC/muscle volume ratio would correlate with increased mTMD pain duration and intensity. However, our results did not demonstrate a correlation between the ATC/muscle volume ratio and pain duration or intensity.

Our cadaveric dissection confirmed the superficial, zygomatic, and deep parts to the temporalis in concordance with Gaudy (2002) and Sedlmayr (2009). We used our cadaveric dissection of the temporalis to inform and guide our MRI segmentation. The high intraclass correlation coefficients indicate that our MRI segmentation method was reliable. Both in the cadaver and on MR images, the deep temporalis was discernible as distinct from the superior head of the lateral pterygoid muscle due to an intervening fat plane, as previously noted (Geers et al, 2005). This boundary made it easier to segment the deep temporalis from the lateral pterygoid muscle and adjacent structures.

Previous studies have investigated masticatory muscle abnormalities in mTMD. These investigations mostly focused on the lateral pterygoid and masseter muscles (Ariji et al, 2004; D'Ippolito et al, 2010; Lopes et al, 2015; Nikkuni et al, 2013; Park et al, 2012; Taskaya-Yilmaz
et al, 2005; Tomas et al, 2006). Three studies reported that the lateral pterygoid muscle is abnormal in TMD (D'Ippolito et al, 2010; Lopes et al, 2015; Taskaya-Yilmaz et al, 2005). However, the methods used may be questioned, and thus caution should be taken when interpreting the results of these studies. D’Ippolito et al. (2010) reported higher axial muscle thickness of the lateral pterygoid muscle in TMD patients. However, they also reported abnormalities in control patients, with altered muscle thickness, atrophy, and contracture (D’Ippolito et al, 2010). These assessments were qualitative in nature. Also, the authors did not investigate the internal architecture of the lateral pterygoid, quantify volumes, nor did they test for differences between the TMD and control groups. Taskaya-Yilmaz et al. (2005) reported, based on subjective interpretations of structural MR images, that the lateral pterygoid muscles were atrophied and degenerated in TMD patients with disc displacement without reduction compared to healthy controls. They also did not quantify muscle volumes, nor test for group differences. Another key difference between these two studies and our study is that the two aforementioned studies did not investigate patients with chronic mTMD. Thus, while these authors report muscle abnormalities in TMDs, they are of limited utility for comparison to our study. Lopes et al. (2015) segmented the lateral pterygoid muscle in two TMD cohorts: with and without migraine. They reported that TMD patients with migraines had larger lateral pterygoid muscles than those with TMD alone (Lopes et al, 2015). However, given that a healthy control group was not included, these data cannot be compared to our cohort.

The masseter, a muscle that is often painful in mTMD patients (Schiffman et al, 2014), has been the subject of several investigations. Ariji et al. (2004) compared the masseters of patients with mTMD with healthy controls using ultrasonography. They scanned the anterior border of the masseter muscle approximately 2.5 cm superior to the inferior border of the mandible at rest and at maximum contraction, measured the maximal masseter thickness, and subjectively evaluated its internal ATC. The authors found thicker masseter muscles in mTMD patients at rest. They also found that a small proportion of mTMD patients exhibited the absence or fewer internal masseter echogenic bands (ATCs), which indicates that ATCs may be less visible in mTMD. The authors attributed the thicker masseter in mTMD to edema, which is an inflammatory change. This result contrasts our finding of no differences in temporalis muscle volumes between chronic mTMD patients and healthy controls. It is possible that, in contrast to acute or subacute mTMD, edema may subside in chronic mTMD. Therefore, muscle volumes would not be affected.
However, whether edema is present only in patients with acute mTMD and not chronic mTMD has yet to be tested. The reduced ATC noted in the masseter is in line with our results. We could further speculate that such abnormalities may precede the onset of pain, and perhaps predispose those affected to developing mTMD. Nevertheless, the complex internal structure of the masseter and the use of different measures (muscle thickness vs. muscle volume) limit comparison of these results with our findings in the temporalis and highlight the need for further characterization of mTMD both in the acute and chronic phases.

Another study investigating masseter structure in mTMD patients using ultrasound reported no differences between mTMD and control groups (Poveda-Roda et al, 2018). They measured masseter width at the mid-point between the inferior border of the mandible and zygomatic arch in light occlusal contact and under maximal muscle contraction. Individuals with chronic mTMD present with greater masticatory muscle activity compared to healthy controls (Suvinen & Kemppainen, 2007; Visser et al, 1995). Interestingly, reduced muscle reoxygenation after exercise has been reported in the masseter of mTMD patients, as well as healthy individuals with increased oral parafunctions (Delcanho et al, 1996; Ferreira et al, 2017). Reduced reperfusion leads to ischemia (Millar et al, 2012; Sharma & Maffulli, 2006). Therefore, muscle hypertrophy or ischemic atrophy may lead to differences in muscle volumes of mTMD patients (as we hypothesized in Aim 2). However, both our study and Poveda-Roda’s study did not find increased muscle volumes and widths in mTMD, respectively. It is possible that the lack of muscle hypertrophy in response to increased functional demands in mTMD may be due to local ischemia. Persistent local ischemia may also contribute to smaller ATC volumes associated with mTMD. However, further studies are needed to verify this proposed mechanism.

The relationship between the ATC and muscle has been used to study tendon injury in the field of sports medicine. A recent study used computational models to investigate whether alterations in the proximal ATC width of the long head of the biceps femoris (hamstring) affected the risk of injury (Fiorentino & Blemker, 2014). They found that a reduced ATC width increased the musculotendon tissue unit strain and thus increased the susceptibility to injury. Therefore, we propose that the smaller temporalis ATC/muscle ratio observed in mTMD groups across both cohorts may predispose these patients to injury. However, further mechanistic studies are needed to demonstrate that a reduced ATC/muscle ratio predisposes the muscles of mastication to injury.
Our study did not demonstrate a correlation between the temporalis ATC/muscle volume ratio and either the mTMD pain duration or intensity. Although it is possible that a correlation does not exist, our results may be partially explained by a limited sample size, missing data, and insufficient data quality. The recall of pain duration and reports of overall pain intensity may have been modulated by psychosocial factors. Further, there were missing pain intensity data from the Sydney cohort. Future studies may use more objective measures, such as pain rating on muscle palpation, to test whether masticatory muscle abnormalities correlate with pain.

An ancillary finding of our study was that mTMD patients had larger superior-inferior skull measurements in the Sydney cohort and greater anterior-posterior skull measurements in the Toronto cohort. These findings closely reflect the dolichocephalic (longer and taller skull) features of many mTMD patients.

These structural abnormalities within the temporalis may be one possible source of nociceptive drive to the trigeminal nociceptive pathway. Muscle injury and persistent inflammation may contribute to a persistent nociceptive barrage, which could explain the abnormalities found along the entire trigeminal nociceptive pathway in chronic mTMD patients (Moayedi et al, 2012b; Wilcox et al, 2015). However, given the cross-sectional nature of our study, we can only speculate such a mechanism, and further investigation is required to establish whether the structural abnormalities found in the temporalis of mTMD patients precede and drive CNV and CNS structural abnormalities. The structural abnormalities found within the temporalis in chronic mTMD may contribute to study novel mechanisms regarding this condition. Our findings add to the current understanding of tendinopathy which suggests a role of ATC atrophy in chronic musculoskeletal pathologic states (Cook & Purdam, 2009; Kaux et al, 2011; Sharma & Maffulli, 2006; Thomopoulos et al, 2015).

Our investigation has some limitations, and some of these have been discussed above. First, the ATC is not discernable from the temporalis muscle within the most cranial portion of the muscle on the T1-weighted MR images. This was corroborated by our dissection, which demonstrated that the temporalis is very thin superiorly. Furthermore, in the most caudal portion of the muscle, at its attachment on the coronoid process, similar MR signal intensities hindered distinction of ATC from cortical bone (both low signals i.e. black/dark). To address this limitation, we limited our characterization of the ATC superiorly by the superior orbital rim and inferiorly by the first
axial slice where signal from cancellous bone of the coronoid process was seen. Thus, it is likely that our segmentation underestimated the ATC volumes in both groups and both cohorts. Therefore, the underestimated ATC volume should have minimally affected the group differences in ATC/muscle volume ratio in both cohorts.

The MRI data from the two different cohorts were acquired with two different scanners, using different imaging parameters. The slightly larger voxel sizes of the Toronto studies may have resulted in increased voxel selection during segmentation. The differences in acquisitions prevented direct comparison and statistical tests between the study cohorts. Therefore, we used the Sydney cohort to determine if there were differences in the ATC/muscle volume ratio and the Toronto cohort served as an independent validation.

6.1 Proposed model

As this is a cross-sectional study, one cannot be sure that the smaller temporalis ATC/muscle volume ratio is a precipitating factor rather than an adaptive response to pain. It may be inferred from studies of other muscles that smaller ATC volumes predisposes susceptible individuals to future injury and thus pain (Fiorentino & Blemker, 2014). The smaller ATC volume is likely a result of inflammation within the muscles of mastication (Jounger et al, 2017). While acute inflammation may result in muscle edema (Ariji et al, 2004), chronic changes likely result in tendon destruction (Cook & Purdam, 2009). Recent findings of reduced oxygen extraction within masticatory muscles may be a consequence of hemodynamic changes (Delcanho et al, 1996; Ferreira et al, 2017). Interestingly, similar findings of lowered blood flow have been reported in trapezius myalgia (Larsson et al, 1999). It is possible that these hypo-oxygenated muscles in a chronic inflammation state exacerbate tissue damage, predisposing the muscle to further injury without a chance to adequately repair (Fiorentino & Blemker, 2014).

Previous studies have shown abnormalities along the trigeminal nerve and the entire trigeminal nociceptive pathway, and had led researchers to hypothesize that there may be increased nociceptive barrage (Moayedi et al, 2012b; Wilcox et al, 2015; Younger et al, 2010). Notably, some of these abnormalities were related to the duration of pain suggesting progressive disease (Moayedi et al, 2011). Using the temporalis as a model, our novel findings reveal that the muscles of mastication are abnormal in mTMD. It is possible that these muscles in mTMD may be one potential source of the peripheral nociceptive barrage.
Chapter 7

7 Conclusions

Patients with chronic mTMD demonstrated similar temporalis muscle volumes and smaller temporalis ATC volumes compared with age- and sex-matched, healthy, pain-free controls across two independent cohorts. Due to smaller ATC volumes, patients with chronic mTMD have smaller ATC/muscle ratios compared with pain-free controls.

7.1 Significance

We have developed a semi-automated segmentation protocol using 3D MRI data to study the human temporalis muscle and ATC. In doing so, we have established an ATC/muscle volume database of mTMD patients and controls. Our observations of a smaller temporalis ATC/muscle ratio in mTMD patients compared with healthy, pain-free controls is novel, identifying a peripheral morphological anomaly in mTMD patients. This anomaly may reflect malfunctioning or poor adaptation of the muscles of mastication, and these changes may contribute to a peripheral nociceptive drive of the trigeminal sensory pathway. While our findings suggest that peripheral muscle abnormalities are associated with CNS abnormalities, further studies are required to establish whether this finding precedes or is the result of chronic pain.
Chapter 8

8 Future Directions

The results of this work are indeed interesting and add to the current knowledge of TMDs. The current study has possible future applications, some of which are discussed below.

To start, the findings of this study will lay the groundwork for a prospective study, testing whether muscular, CN V and CNS abnormalities are progressive in mTMD. Also, imaging protocols should be investigated that most ideally allow for optimal delineation of the temporalis muscle and ATC. Finally, investigating the other muscles of mastication for other changes in mTMD may also be useful.

Future investigations of normal temporalis development with craniofacial growth would be helpful in determining whether the ATC/muscle volume ratio is stable over time. In addition, the role of hormonal factors in mTMD could be investigated as well as the impact of these factors on the ATC/muscle volume ratio. If ATC/muscle volume ratio does not change with growth and ageing, and predicts mTMD severity, it may be considered a specific sign of the condition and may help develop novel management strategies.

In the long-term, we hope that the structural abnormalities described in this thesis may be used to understand the underpinnings of mTMD, track its progression, and inform us about disease.
References


Weijs, W. & Hillen, B. (1986) Correlations between the cross-sectional area of the jaw muscles and craniofacial size and shape. *Am J Phys Anthropol* (0002-9483 (Print)).


Figure 21: Comparison of repeatability of different segmentation methods of the right 
temporalis muscles. Triplicate measurements using four different segmentation methods 
without refinement. Measurements (in mm$^3$) provided below. This analysis suggests that 
segmentation in one plane alone overestimates volume measures with greater variability 
compared to auto-segmentation.

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Table 7: Triplicate measurements of eight randomly selected MRI studies from the Sydney data.

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