Research Paper

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Structural abnormalities in the temporalis musculo-aponeurotic complex in chronic muscular temporomandibular disorders

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Abstract

Some forms of chronic pain are thought to be driven and maintained by nociceptive input, which can drive plasticity within nociceptive pathways. We have previously identified abnormalities along the entire nociceptive pathway in chronic myalgic temporomandibular disorders (mTMD), including the trigeminal nerves, brainstem pathways, and in the thalamus and somatosensory cortex. These data suggest that there is a peripheral nociceptive drive in mTMD, but the source of this nociceptive activity remains unknown. Here, our aim was to determine whether structural abnormalities exist in the muscles of mastication of patients with chronic mTMD. Specifically, we tested whether the volume of the temporalis muscle and its tendon–aponeurosis complex (TAC, a structure that dissipates forces in a muscle) in mTMD patients differ compared to age- and sex-matched controls. To do so, we segmented these structures on T1-weighted structural magnetic resonance images. We found that muscle volumes in mTMD were not different to controls. However, the mTMD group had significantly smaller volumes of the bilateral temporalis TAC, and thus a smaller TAC-to-muscle volume ratio. These findings were consistent across 2 independent cohorts of 17 mTMD patients, compared to 17 age- and sex-matched controls. We propose a model where reduced TAC-to-muscle ratio could result in a predisposition to muscle tissue injury. In sum, abnormalities of the temporalis muscles in mTMD supports our hypothesis that chronic mTMD pathophysiology may be related to peripheral nociceptive barrage originating from the muscles of mastication.

Keywords: Temporomandibular disorders, Muscle, Masticatory muscles, Temporalis muscle, MRI, Tendon, Myogenic TMD, Imaging

1. Introduction

Temporomandibular disorders (TMDs) affect the temporomandibular joint, the masticatory musculature, and/or related structures.[20,32] Temporomandibular disorders are the most common orofacial chronic pain disorder,[12] affecting between 10% and 20% of the population,[9,22,23,26] mostly women.[3,7,11,12,16,36] Some forms of TMDs are painful, with most complaints being muscular in nature (TMD myalgia, myalgic temporomandibular disorders [mTMD]).[19] The underlying pathophysiological mechanisms and etiological factors that contribute to the development of mTMD are not fully understood.[10,13,14,33]

We and others have previously shown structural abnormalities along the entire trigeminal nociceptive pathway in chronic mTMD.[28,30,45,46] The trigeminal nerves were abnormal in mTMD, related to the duration of TMD symptomatology, suggesting that the abnormalities were driven by ongoing pain and related symptoms.[30,45] We also found widespread reductions in brain white matter integrity, including in the brainstem, as well as thalamocortical tracts projecting to somatomotor cortices.[30] Myalgic temporomandibular disorders patients also have abnormal gray matter in the brainstem, thalamus, and S1 cortex.[28,46] Finally, we found a correlation between thalamic gray matter and the duration of pain. Together, structural changes, which include changes from the peripheral nerve to the cortex, are consistent with the idea that mTMD is associated with ongoing increased nociceptive drive to the central nervous system. The source of such a peripheral afferent barrage into the central nervous system is unknown.

One potential source of the nociceptive drive in mTMD may be the painful masticatory muscles. Convergent evidence indicates that patients with mTMD have abnormal masticatory muscle function. Specifically, patients with chronic mTMD have reductions in oxygen saturation in the masticatory muscles, and abnormal muscular blood flow.[8,15] Similarly, healthy individuals who have high rates of clenching (a strong risk for mTMD) also had abnormal muscular blood flow and oxygenation.[39] These

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oxygenation abnormalities can lead to ischemia, and thus induce tissue injury[43] and inflammation.[25,42]

The masticatory muscles include the temporalis, the masseter, and the lateral and medial pterygoids. The temporalis is a large muscle located in the infratemporal fossa that has 2 compartments, the superficial and deep temporalis, divided by a large fan-shaped aponeurosis that forms the temporalis tendon–aponeurosis complex (TAC) (**Fig. 1**). Aponeuroses are fibrous sheets originating from tendons that attach sheet-like muscles. The simple geometry of the temporalis muscle and TAC make them suitable for investigation of putative structural abnormalities of muscle in patients with mTMD. For example, a reduced tendon–muscle ratio can negatively influence muscle contraction dynamics and dissipation of energy strains thereby increasing risk of injury.[2,17,31]

The aim of this study was to determine whether patients with chronic mTMD have structural abnormalities in the muscles of mastication. Specifically, we measured temporalis muscle and TAC volumes from magnetic resonance imaging (MRI) scans of patients with chronic mTMD and TMD-free controls. We also calculated TAC to muscle volume ratios. Next, we confirmed our findings in an independent cohort of patients with chronic mTMD. Finally, we tested whether structural abnormalities are related to TMD pain intensity and duration. We hypothesize that patients with chronic mTMD have smaller temporalis TAC, related to TMD duration.

2. Materials and methods

This study investigated 2 previously acquired independent data sets of patients with chronic mTMD, each with their own sex- and age-matched controls. One data set was collected at the University of Sydney, Sydney, Australia (referred to as Sydney cohort herein), and the other at the University Health Network and University of Toronto, Toronto, Canada (referred to as Toronto cohort herein). The MRI scans of patients with TMD and controls were previously acquired by the 2 independent research teams in Canada and Australia to investigate trigeminal nerve and brain abnormalities.[21,28–30,44,45] Both data sets captured the entire temporalis muscle and TACs, bilaterally, from the skull vertex to the level of the sigmoid notch of the mandible.

Local research ethics boards (The University Health Network and Mount Sinai Hospital Research Ethics Boards, Toronto, Canada, and the Westmead Hospital and the University of Sydney Human Research Ethics Committee, Sydney, Australia) approved the studies for which the data sets were collected, and are referred to in previous publications.[21,28–30,44,45] The University of Toronto's Health Sciences Research Ethics Board approved all procedures in this study. For this study, the 2 cohorts were treated as independent, and not pooled.

2.1. Participants

2.1.1. Sydney cohort

All study participants were recruited at the Faculty of Dentistry, Westmead Hospital, University of Sydney (Sydney, New South Wales, Australia). An investigator (I.C.) randomly selected 17 scans of patients with TMD (mean age ± SD: 41.8 ± 16.2 years, 5 men) and 17 controls (mean age ± SD: 42.0 ± 17.0 years, 5 men) from the MRI data set pertaining to a previous study that explored brainstem anatomy using anatomical MRI techniques,[45] which included 22 patients with TMD and 40 TMD-free controls. The selection of controls was made to age- and sex-match the patient group. The RDC-TMD[12] was used to make TMD diagnosis.

As per the previous investigation,[45] patients from the Sydney cohort had no neuropathic pain symptoms or other chronic pain conditions. However, they could participate in the study if they had other nonchronic musculoskeletal pain complaints in other body regions or infrequent migraines, based on their medical history. Controls were screened by self-report for exclusion criteria including chronic pain (pain lasting for more than 3 months), current usage of analgesic medications, or any neurological disorders. Demographic and clinical characteristics of patients in the Sydney cohort are reported in **Table 1**.

2.1.1.1. Magnetic resonance imaging parameters

Data were acquired using a 3.0 T MRI system (Philips; Intera, De Bilt, Netherlands). High-resolution, three-dimensional (3D), T1-weighted anatomical image volumes were acquired, covering the entire brain and included the entire temporalis muscle (turbo field echo; echo time: 2.5 ms; repetition time: 5600 ms; flip angle: 8°; voxel dimensions: 0.8 × 0.8 × 0.8 mm).

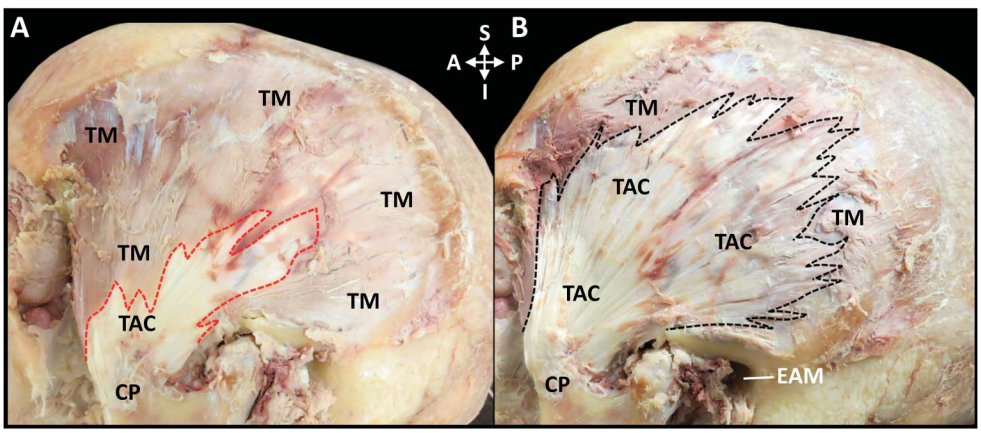

Figure 1. Anatomical verification. Dissection of tendon–aponeurosis complex of temporalis muscle (TAC), lateral view. (A) Superficial dissection showing extramuscular part of the TAC. Red dashed lines indicate the fiber bundle–TAC junction. (B) Deep dissection showing extramuscular and intramuscular extent of TAC. Black dashed line indicates fiber bundle TAC junction. CP, coronoid process; EAM, external auditory meatus; TAC, tendon–aponeurosis complex; TM, temporalis muscle.

Table 1

Clinical and demographic characteristics of the Sydney cohort of patients withTMD.

Age	Sex	TMD dur (y)	Diary pain intensity (at rest, /10)	Scan pain intensity (at rest, /10)	Diagnosis (RDC/ TMD)	Pain sites	Previous trauma	Other pains	Medications
59	M	20	5.9	6	Bilateral MFP Pain localised to the hard palate	RM, LM			Amitriptyline
59	F	30	7.9	7.6	Bilateral MFP	RM, LM			Venflaxine, Acetaminophen and codeine, Oxycodone, Tramadol
70	F	11	0.6	4.6	Bilateral MFP Left Arthralgia	RM, LM, RTMJ, LTMJ	Y	Shoulder, knee	Atorvastatin calcium, Strontium ranelate, Diltiazem Hydrochloride
37	M	6	7.3	7	Bilateral MFP Bilateral Arthralgia	RM, LM, RT, LT	Y	Neck	Botox
50	F	46	4.3	2.7	Bilateral MFP	RM, LM, RTMJ, LTMJ	Y	Shoulder, neck	Diclofenac, Botox (forehead), Acupuncture magnets, Acetaminophen and codeine
45	F	5.5	4.1	7.3	Bilateral MFP	RM, LM			Acetaminophen, Acetaminophen and codeine, Oxycodone, Mometason (nasal spray), Mersyndol
32	F	15	5.9	5.5	Right MFP	RM		Shoulder, neck	Acetaminophen, codeine phosphate hemihydrate, doxylamine succinate (Mersyndol)
28	M	1.5	5.8	6.7	Bilateral MFP Right Arthralgia	RM, LM	Y		Ibuprofen
24	F	7	5.5	5.9	Bilateral MFP with LMO Bilateral Arthralgia	RM, LM	Y		Domperidone, Acetaminophen, Lansoprazole, Levlen, Amitriptyline
66	F	5	3.1	3.7	Right MFP	RM		Shoulder, neck	
31	F	4	2.2	5.5	Left MFP	LM		Lower back	
55	F	18	1.5	0.4	Right MFP Right Arthralgia	RM		Shoulder	
45	F	12	3.2	6	Bilateral MFP	LM, LT		Shoulder	Amitriptyline, Acetaminophen
44	F	17	1.2	2.6	Bilateral MFP Bilateral Arthralgia Right disc displacement with reduction	RM, LM		Lower back	Ibuprofen, Acetaminophen
19	F	4	3.8	4.5	Bilateral MFP (with neck pain)	RM, LM, RT, LT	Y	Neck	Acetaminophen
19	M	2.5	3.1	1	Bilateral MFP (with neck pain) Bilateral arthralgia	RM, LM, RT, LT			
28	M	3	5.9	3.5	Bilateral MFP (with neck pain) Bilateral arthralgia	RM, LM, RT, LT	Y		Aspirin/caffeine/ orphenadrine (Norgesic), meloxicam
Mean (SD)		12.2 (11.6)	4.2 (2.1)	4.7 (2.2)					

LM, left masseter; LOM, limited mouth opening; LT, left temporalis; LTMJ, left temporomandibular joint; MFP, myofascial pain; RM, right masseter; RT, right temporalis; RTMJ, right temporomandibular joint; Y, yes .

2.1.1.2. Pain characteristics

Patients with mTMD reported the duration of their TMD symptoms, and provided a pain intensity rating on the day of their MRI scan (scan day pain intensity). In addition, patients completed a pain diary for 7 days before scanning, and recorded their pain intensity 3 times a day (diary pain intensity). Pain drawings were recorded, and each patient indicated the location of facial pain. Pain characteristics (intensity and duration), and location (based on pain drawings) of patients in the Sydney cohort are reported in **Table 1**.

2.1.2. Toronto cohort

Patients were recruited at the Mount Sinai Hospital Dental Clinic (Toronto, ON, Canada), and controls from the University Health Network environment. Seventeen women with mTMD (mean age ± SD = 33.1 ± 11.9 years) were diagnosed using the RDC-TMD,[12] and 17 age-matched pain-free women (mean age ± SD = 32.8 ± 9.8 years) were recruited. This cohort was previously recruited for studying brain and trigeminal nerve abnormalities in TMD. Inclusion and exclusion criteria for those studies were reported previously[21,28–30,44] and included: (1) left-handedness; (2) self-report of metabolic, rheumatoid or vascular diseases/disorders, or any other serious diseases; (3) self-report of commonly comorbid functional chronic pain disorders (irritable bowel syndrome and fibromyalgia); (4) self-report of psychiatric disorders (eg, depression, schizophrenia); (5) self-report history of an abnormal neurological examination; (6) contraindication to MRI scanning; and (7) self-report of substance abuse. In addition, healthy controls were excluded if they had a history of chronic pain or any other vascular or neurological disorders, based on self-report. Demographic and clinical characteristics of patients in the Toronto cohort are reported in **Table 2**.

2.1.2.1. Magnetic resonance imaging parameters

The Toronto data set was acquired using a 3.0 T MRI system (GE Signa HDx, General Electric) with an eight-channel phased-array head coil. High-resolution 3D T1-weighted anatomical images were acquired, covering the entire brain and included the entire temporalis muscle (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 mm).

2.1.2.2. Pain characteristics

Patients with mTMD reported the duration of their TMD symptoms, and provided a verbal numerical pain intensity score for their average pain intensity over the last month before scanning. Pain drawings were recorded, and each patient indicated the location of facial pain. Pain characteristics (intensity and duration), and location (based on pain drawings) of patients in the Toronto cohort are reported in **Table 2**.

2.2. Anatomical verification

To identify anatomical landmarks of the temporalis muscle and TAC to guide our MR segmentations, a dissection of a formalin-embalmed hemisected head of a 79-year-old man was performed (**Fig. 1**). The skin and subcutaneous tissue were removed to expose the temporalis fascia, masseter, and lateral aspect of cranium. The masseter was released from its attachment sites and removed. Next, the zygomatic arch was excised to expose the temporalis fascia and underlying temporalis muscle in its entirety. After careful removal of the temporalis fascia, the superficial temporalis and inferior part of the TAC attaching to the coronoid process could be visualized. The fiber bundles of superficial temporalis were delineated, their extent documented, and photographed. This was followed by removal of the fiber bundles to expose the entire TAC complex and fiber bundles of the deep temporalis, which attached inferiorly to the deep surface of the TAC (**Fig. 1**). The terminal attachment of the tendinous component of TAC was to 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. The extensive aponeurotic part of the TAC became thin and poorly discernible superior to the superior orbital rim.

2.3. Magnetic resonance regions of interest definition

Based on the anatomical study (**Fig. 1**), the superior boundary of the TAC region of interest (ROI) in MR images was set at the level of the superior orbital rim (**Fig. 2**). The inferior boundary was set to the first axial slice where the higher signal cancellous bone within the coronoid process of the mandible was visualized (**Fig. 2**). The anterior, posterior, medial, and lateral boundaries of the TAC ROI were the anterior, posterior, medial, and lateral surfaces of the muscle, respectively.

The temporalis muscle ROI included the entire muscle, and was limited inferiorly at the first axial MRI slice where the higher signal cancellous bone within the coronoid process of the mandible was visualized.

2.4. Quantification of temporalis muscle and tendon–aponeurosis complex

User-guided segmentation of the temporalis muscle and TAC was performed in ITK-SNAP (www.itksnap.org),[47] with a Huion WH1409 (Shenzhen, China) graphic tablet. All images were segmented by a Resident in Oral and Maxillofacial Radiology (G.K.), who also conducted the anatomical dissection. The operator was blinded as to the group assignment of participants from each study cohort. Each temporalis muscle and TAC was segmented independent of each other and the contralateral using a semiautomatic segmentation pipeline. Information from the T1-weighted MR images was reduced to a single foreground/background probability map. ROIs of the structures were created in a semiautomatic way, and manually refined (**Fig. 3**). Each MR image was segmented in the following order: left TAC, left temporalis muscle, right TAC, and right temporalis muscle. After the first round of segmentations, each MR study was verified in all orthogonal planes after a one-week washout. Any disagreement was resolved after discussion with an expert anatomist (A.A.) and an expert in masticatory muscle MR imaging (I.C.), who were also blinded to group assignment. The volumetric data were then recorded for analysis.

2.4.1. Intrarater reliability

To test the reproducibility of the segmentation method, within the *Sydney* cohort, the ROIs of 8 randomly selected participants (4 controls and 4 mTMD) were segmented a second time 1 month after the initial segmentation. Intraclass correlation coefficients (ICCs) were calculated to compare the measurements of muscle and TAC volumes to measure intrarater reliability (see below).

Table 2

Clinical and demographic characteristics of the Toronto cohort of patients with TMD.

Age	Sex	TMD dur (y)	PAIN intensity (at rest/10)	Diagnosis (RDC/TMD)	Pain sites	Previous trauma	Other pains	Medications
22	F	2	4	Bilateral MFP Bilateral arthralgia	RT, RTMJ, LT, LTMJ		Knee	NSAIDs
20	F	3	3	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, RTMJ, LTMJ			Diclofenac and misoprostol, cyclobenzaprine
24	F	7	4	Right MFP with LMO Right arthralgia	RM, RTMJ	Y	Headache	Naproxen, lansoprazole
38	F	20	7	Bilateral MFP with LMO Bilateral arthralgia	RM, LM, RTMJ, LTMJ			NSAIDs
42	F	0.75	2	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, LM, LT, RTMJ, LTMJ			Cyclobenzaprine
33	F	4	5	Bilateral MFP with LMO Right arthralgia	RM, RT, RTMJ, LM, LT		Shoulder, neck	
28	F	17	3	Left MFP Left arthralgia	LM			Diclofenac and misoprostol, cyclobenzaprine
34	F	14	2	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, RTMJ, LM, LT, LTMJ			Diclofenac and misoprostol, cyclobenzaprine, hydromorphone
50	F	10	7	Bilateral MFP with LMO Bilateral arthralgia	RM, RTMJ, LM, LTMJ	Y		Diclofenac and misoprostol Varenicline tartrate, clonidine hydrochloride
59	F	13	7	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, RTMJ, LM, LT, LTMJ	Y	Neck, shoulder, gastric distress	
18	F	3	6	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, RTMJ, LM, LT, LTMJ			
34	F	15	2	Right MFP with LMO	RM, RT		Neck, sciatica	
52	F	30	4	Bilateral MFP with LMO Bilateral arthralgia	RM, RT, RTMJ, LM, LT, LTMJ			NSAIDs
31	F	2	6	Right MFP with LMO Right arthralgia	RM, RT, RTMJ		Ovarian cyst	NSAIDs
33	F	17	5	Bilateral MFP with LMO Bilateral arthralgia	RM, RTMJ, LM, LTMJ			NSAIDs
22	F	1	4	Bilateral MFP Bilateral arthralgia	RM, LM, RTMJ, LTMJ			

(*continued on next page*)

Table 2 (*continued*)

Age	Sex	TMD dur (y)	PAIN intensity (at rest/10)	Diagnosis (RDC/TMD)	Pain sites	Previous trauma	Other pains	Medications
23	F	8	2	Bilateral MFP Bilateral arthralgia	RM, RTMJ, LM, LTMJ			
Mean (SD)		9.8 (8.2)	4.3 (1.8)					

LM, left masseter; LOM, limited mouth opening; LT, left temporalis; LTMJ, left temporomandibular joint; MFP, myofascial pain; RM, right masseter; RT, right temporalis; RTMJ, right temporomandibular joint; Y, yes.

2.5. Statistical analyses

All statistical analyses were performed separately for the Sydney and Toronto cohorts because they were considered to be independent of one another.

Intraclass correlation coefficients were computed to measure intrarater reliability for muscle and TAC volumes. Intraclass correlation coefficients less than 0.4, between 0.4 and 0.75, and greater than 0.75 are considered poor, fair to good, and excellent, respectively.

To test for group differences in muscle and TAC volumes, 2 multivariate analyses of variance were used, one for each cohort (Sydney and Toronto). Because data were not normally distributed, volumetric data were logarithmic transformed (using a natural logarithm). In each multivariate analysis of variance, temporalis muscle and TAC volumes were included as dependent variables, whereas group (mTMD vs control), laterality (left vs right), and the group-by-laterality interaction were included as independent variables. Significance was set at $P < 0.05$, corrected for multiple comparisons using Bonferroni correction, where applicable.

For each cohort, we also computed a TAC volume to muscle volume ratio (*TAC:muscleR*). Evidence suggests that reduced tendon–muscle ratio can negatively influence muscle contraction dynamics and force dissipation, increasing the risk of injury.[2,17,31] Thus, this measure was calculated to determine whether the relative proportion of TAC was reduced in the muscles of patients with mTMD as compared to healthy controls. To test for group differences in the TAC:muscleR, 2 univariate analyses of variance were used, one for each cohort (Sydney and Toronto). TAC:muscleR was included as the dependent variable, whereas group (mTMD vs control), laterality (left vs right), and the group-by-laterality interaction were included as independent variables. Significance was set at $P < 0.05$, corrected for multiple comparisons using Bonferroni correction, where applicable.

To determine the contribution of pain characteristics to abnormalities in mTMD, Spearman correlation coefficients between the left and right temporalis muscle volumes, TACs, and TAC:muscleR and (1) mTMD duration and (2) pain intensity (diary pain intensity for the Sydney cohort and the average pain intensity over the past month in the Toronto cohort) were computed. Significance was set at $P < 0.05$. All statistical analyses were performed in SPSS Statistics for Windows (Version 24.0, IBM, Armonk, NY).

3. Results

3.1. Intraclass correlations

In the 8 randomly selected subjects who were bilaterally segmented in duplicates 1 month after initial segmentation, the intrarater reliability was excellent for both the temporalis muscle and the TAC (*muscle:* Cronbach alpha = 0.992; ICC: 0.98, 95% CI = 0.96-0.99; *TAC*: Cronbach alpha = 0.963, $P < 0.001$; ICC: 0.93; 95% CI = 0.82-0.98, $P < 0.001$).

3.2. Group statistics

There were no significant age differences between mTMD and controls in either the Sydney (P = 0.972) or the Toronto ($P = 0.936$) cohorts. We tested whether there were significant

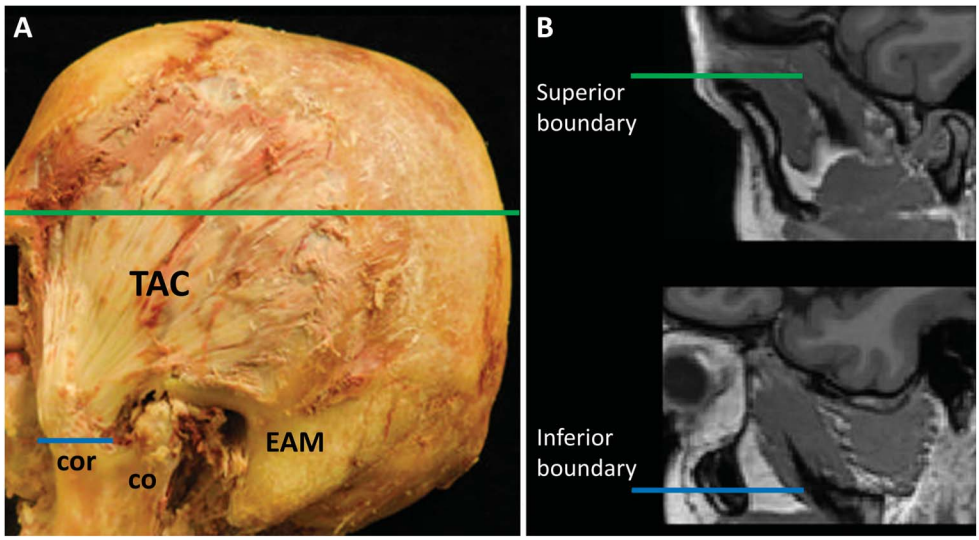

Figure 2. Tendon–aponeurosis complex (TAC) region of interest definition. (A) Upper (green) and lower (blue) boundaries of the TAC region of interest in the cadaver. (B) Upper and lower boundaries of the TAC region of interest in MR image (sagittal plane).

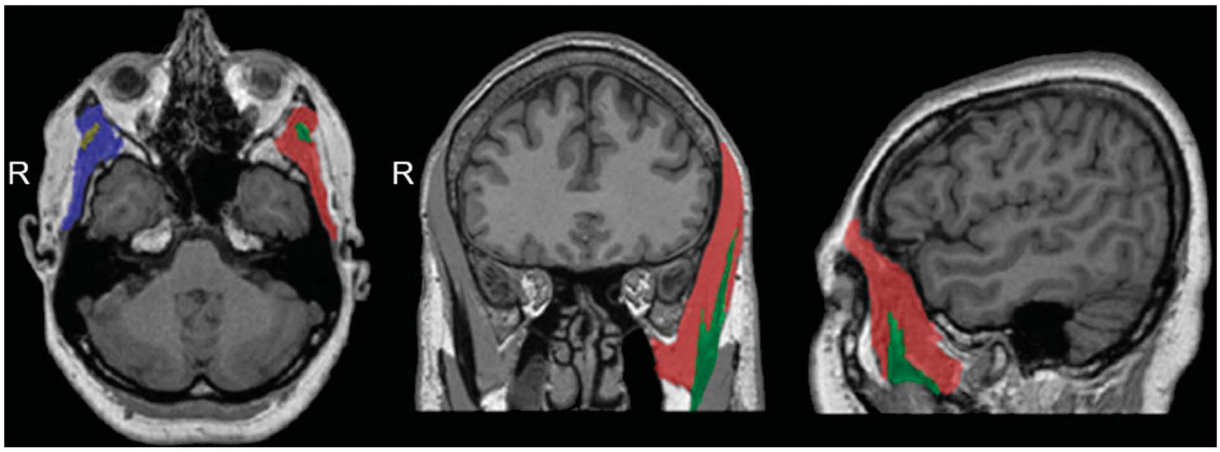

Figure 3. MR segmentation. Segmented axial, coronal, sagittal images of the left temporalis muscle (red) and its TAC (green) in one typical participant, and the right temporalis muscle (blue). R, right side; TAC, tendon–aponeurosis complex.

differences in temporalis muscle and TAC volumes between participants with mTMD vs pain-free control. We did not find an effect of study group (mTMD vs controls) on the temporalis muscle volumes in both the Sydney (Mean ± SD; Controls = 33466.48 ± 6809.27 mm^3, mTMD = 31462.22 ± 8195.78 mm^3; $F_{2, 63} = 1.652$, $P = 0.203$; Wilk $\Lambda = 0.837$, partial $\eta^2 = 0.025$; **Table 3A** and **Fig. 4A**) and Toronto (Mean ± SD; Controls = 31534.67 ± 6592.86 mm^3, Patients = 31192.95 ± 7602.19 mm^3; $F_{2, 63} = 0.092$, $P = 0.763$; Wilk $\Lambda = 0.671$, partial $\eta^2 = 0.001$; **Table 3B** and **Fig. 4B**) cohorts.

Next, we sought to determine whether there were group differences in temporalis TAC volumes. Participants with chronic mTMD had significantly smaller temporalis TAC volumes, compared to pain-free healthy controls in both the Sydney (Mean ± SD; Controls = 2092.49 ± 498.20 mm^3, mTMD = 1633.52 ± 641.27mm^3; $F_{2, 63} = 12.368$, $P = 0.001$; Wilk $\Lambda = 0.837$, partial $\eta^2 = 0.162$; **Table 3A** and **Fig. 4A**) and Toronto (Mean ± SD; Controls = 3910.41 ± 989.93 mm^3, mTMD = 2808.85 ± 713.09 mm^3; $F_{2, 63} = 29.601$, $P < 0.001$; Wilk $\Lambda = 0.671$, partial $\eta^2 = 0.316$; **Table 3B** and **Fig. 4B**) cohorts.

Patients with mTMD had a smaller temporalis TAC:muscleR compared with controls in both the Sydney (Mean ± SD; Controls = 6.40 ± 1.74%, mTMD = 5.28 ± 1.77%; $F_{2,63} = 6.981$, $P = 0.010$; **Table 4A** and **Fig. 4A**) and Toronto (Mean ± SD; Controls

Table 3

Multivariate analysis of variance (MANOVA).

MANOVA	Dependent variable	*df*	Wilk's Λ	F	*P*
A. Sydney cohort					
Corrected model					
	Muscle	2, 63	<0.001	0.825	0.485
	TAC	2, 63		4.566	**0.006**
Group					
	Muscle	2, 63	0.837	1.652	0.203
	TAC	2, 63		12.368	**0.001**
Side					
	Muscle	2, 63	0.974	0.010	0.922
	TAC	2, 63		1.268	0.264
Group × Side					
	Muscle	2, 63	0.980	0.815	0.370
	TAC	2, 63		0.060	0.807
MANOVA	**Dependent variable**	***df***	**Wilk's Λ**	**F**	***P***
B. Toronto cohort					
Corrected model					
	Muscle	2, 63	<0.001	0.090	0.965
	TAC	2, 63		10.245	**<0.001**
Group					
	Muscle	2, 63	0.671	0.092	0.763
	TAC	2, 63		29.601	**<0.001**
Side					
	Muscle	2, 63	0.985	0.177	0.676
	TAC	2, 63		0.531	0.469
Group × Side					
	Muscle	2, 63	0.990	0.001	0.979
	TAC	2, 63		0.602	0.441

Logarithmically transformed data (temporalis muscle and TAC volumes) from the A. Sydney and B. Toronto cohorts. Bold indicates statistically significant at $P < 0.05$.
df, degrees of freedom.

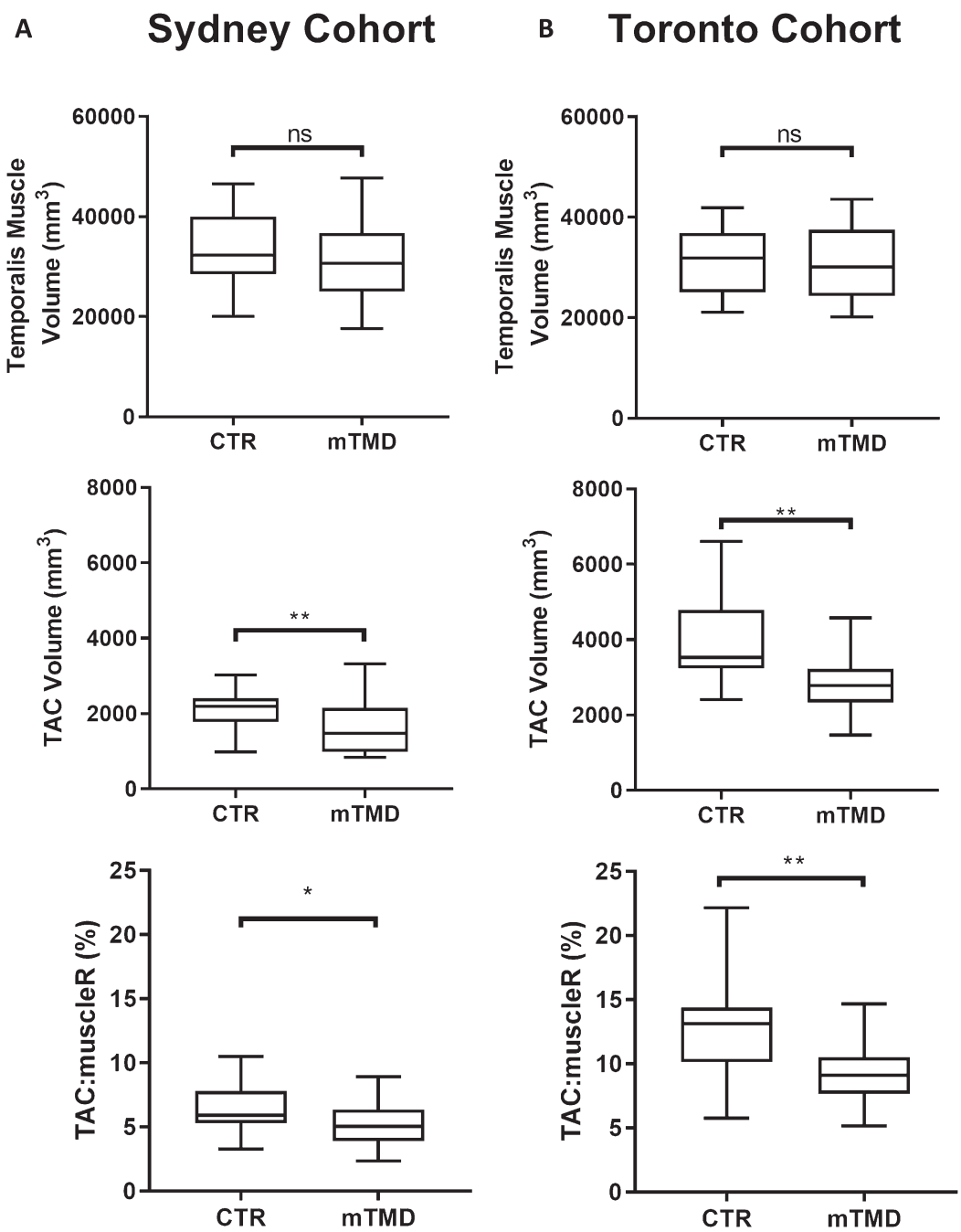

Figure 4. Group comparisons of temporalis muscle, tendon-aponeurosis complex (TAC), and TAC:muscle ratio (%). Group differences between patients with mTMD from (A) the Sydney cohort and (B) the Toronto cohort. The whisker plots represent the median of each group, and the bars represent the maximum and minimum values in each group. Both the Sydney and Toronto cohorts had smaller TAC volumes, and TAC:muscle ratio, compared to healthy age- and sex-matched controls. *$P < 0.05$; **$P < 0.001$. mTMD, myalgic temporomandibular disorders.

= 12.91 ± 3.95%, mTMD = 9.28 ± 2.41%; $F_{2,63} = 20.167$, $P < 0.001$; **Table 4B** and **Fig. 4B**) cohorts.

In the Sydney cohort, there was a significantly negative correlation between the left temporalis muscle volume and the duration of TMD symptoms ($r = -0.61$, $P = 0.009$). There were no other significant correlations for either the Sydney or Toronto cohorts (**Table 5**).

4. Discussion

The aim of this study was to determine whether there are structural abnormalities in the temporalis muscles of patients with chronic mTMD. We investigated the temporalis muscle because of its relatively simple internal architecture, with a single and large TAC. We performed a cadaveric dissection to confirm our ability to image the superficial, zygomatic, and deep parts to the temporalis accurately and in accordance with previous studies,[18,38] which informed our MRI segmentation. We show for the first time that patients with chronic mTMD have smaller TAC volume, which results in a smaller TAC:muscleR because overall muscle volume is normal. Notably, the results were consistent across 2 independent cohorts of patients with chronic mTMD, compared to their respective controls, collected in Canada and Australia. However, in the Sydney cohort, we found that the left

Table 4

Univariate analysis of variance (ANOVA) of the temporalis TAC:muscleR.

Dependent variable: ratio	*df*	F	*P*
A. Sydney cohort			
Corrected model	3	3.338	**0.025**
Group	1	6.981	**0.010**
Side	1	1.793	0.185
Group × Side	1	1.240	0.270
Dependent variable: ratio	***df***	**F**	***P***
B. Toronto cohort			
Corrected model	3	7.182	**<0.001**
Group	1	20.167	**<0.001**
Side	1	0.950	0.333
Group × Side	1	0.001	0.516

Logarithmically transformed data from the A. Sydney and B. Toronto cohorts were used. Bold indicates statistically significant at $P < 0.05$.
df, degrees of freedom.

temporalis muscle volume was significantly negatively correlated with TMD duration, but this finding was not reproduced in the Toronto cohort. In addition, we did not find any correlations with pain intensity in either cohort.

Previous studies have investigated abnormalities in masticatory muscles in patients with mTMD. These investigations focused principally on the lateral pterygoid and masseter muscles. Three studies reported abnormalities in the lateral pterygoid muscles in patients with mTMD,[6,24,41] but methodological limitations limited their interpretability, and they did not formally test group differences between those with TMD and TMD-free controls. Furthermore, none of the studies investigated patients with chronic mTMD, which limits their comparability with our study. With respect to the masseter, one study using ultrasound reported no differences between mTMD and control groups in masseter width,[35] which is consistent with our findings in the other muscles under study in this investigation. However, in another that relied on ultrasound measurements, thicker masseter muscles were observed in patients with mTMD both at rest and during contraction as compared to TMD-free controls.[1] They also reported the absence of or fewer internal masseter echogenic bands (TACs) in a small proportion of patients with mTMD,[1] which indicates that TACs may be less visible in patients with mTMD. The authors attributed the thicker masseter in patients with mTMD to the presence of edema, but could not confirm this with ultrasound. The larger masseter muscle result is in contrast to our findings, whereas the reduced TACs corroborate our findings. Nevertheless, the complex internal structure of the masseter and the use of different measures (muscle width vs muscle volume), and imaging techniques (ultrasound vs MRI) limit comparison of these results with our findings in the temporalis and highlight the need for further multimodal characterization of masseter muscles in mTMD.

Although our study demonstrates differences in TAC volumes between patients with chronic mTMD and controls, the cross-sectional design of our study did not allow us to determine whether TAC abnormalities predate the onset of TMD pain or are sequelae of the disease. The absence of a correlation with pain duration with the TAC volumes or TAC:muscleR in our study likely suggests that TAC structural abnormalities might not be related to progression of disease or may stabilize early on in the disease process. Furthermore, the lack of relationship between ongoing pain intensity and structural abnormalities found in this study may result from a combination of altered peripheral nociceptive drive and altered central mechanisms because it is known that individuals with TMD display enhanced central sensitivity to noxious inputs.[37]

The TAC structural abnormalities could, in part, explain the functional abnormalities described previously in mTMD. Indeed, individuals with chronic mTMD present with greater masticatory muscle activity compared to healthy controls,[4] and have reduced masticatory muscle reperfusion after exercise.[8] Similarly, reduced masticatory muscle oxygenation has been reported in the masseter of healthy individuals at risk of developing mTMD.[39] Reduced reperfusion leads to hypoxia, inflammation, increased oxygen radical production, and injury of muscle tissue.[5,34,43] Although muscle hypoxia has been reported to be a critical pathophysiological determinant of tendon injury in other body regions,[27,40] this is yet to be determined in the temporalis muscle, and the concurrence of such abnormalities is yet to be tested in TMD.

Abnormalities in TAC volumes affect the ability of the muscle to dissipate forces generated during function. Using computational models, Fiorentino and Blemker showed that alterations in the proximal TAC width of the long head of the biceps femoris (hamstring) determine changes in the strains within the muscle. In particular, they found that a reduced proximal TAC width increased the musculotendineous tissue unit strain, which can potentially increase the risk of injury.[17] Although this has not yet been tested in masticatory muscles, we hypothesize that the smaller temporalis TAC:muscleR observed in patients with mTMD may preexist the onset of mTMD, and predispose these patients to muscle injury.

Conversely, it is equally feasible that mTMD leads to TAC atrophy, and this is exacerbated with prolonged pain. Accordingly, our data indicate that the left temporalis muscle volume is

Table 5

Spearman's correlations between temporalis muscle volume, TAC volume, and TAC:muscleR and pain characteristics.

	Left		Right		L TAC:muscleR	R TAC:muscleR
	Muscle	TAC	Muscle	TAC		
A. Sydney cohort						
Duration	**−0.61***	−0.28	−0.36	−0.30	0.30	−0.10
Intensity (D)	0.29	−0.24	0.37	0.31	0.27	0.35
	Left		**Right**		**L TAC:muscleR**	**R TAC:muscleR**
	Muscle	**TAC**	**Muscle**	**TAC**		
B. Toronto cohort						
Duration	0.328	**0.50**	0.39	0.31	−0.02	−0.06
Intensity	0.275	−0.24	−0.08	−0.34	−0.27	−0.33

A. Correlations of left (L) and right (R) temporalis muscle volumes, TAC, and TAC:muscleR with TMD duration and diary pain scores (Intensity D) for the Sydney cohort. B. Correlations of L and R temporalis muscle volumes, TAC, and TAC:muscleR with TMD duration and average pain intensity scores over the past month for the Toronto cohort.* Statistically significant ($P = 0.009$).

negatively correlated with TMD duration in the Sydney cohort, suggesting that prolonged pain could have an adverse effect on temporalis TAC volume. However, this finding was not replicated in the Toronto cohort, and thus must be interpreted with caution. We and others have shown previously the presence of structural abnormalities along the trigeminal nociceptive pathway in mTMD.[28,30,45,46] Notably, as previously reported in the Toronto cohort, trigeminal nerve and thalamic abnormalities were related to TMD pain duration,[28,30] suggesting a persistent nociceptive barrage that drove plasticity along the trigeminal nociceptive pathways.[30] One possible source of this nociceptive drive could be the muscle injury and persistent inflammation related to the abnormalities described in the current study. However, given that this study was retrospective and cross-sectional, future studies should aim to disentangle these 2 proposed, competing mechanisms.

Our investigation has limitations, some of which have been discussed above. First, our study included mostly women. The limited number of male participants in the Sydney cohort did not allow us to test sex differences in the TAC and temporalis muscle structure. Second, our study had a relatively small sample size. However, the replication of the effect—namely that TAC volumes were smaller in mTMD, compared to controls—indicates that the study did not suffer from low power. Indeed, the observed effect size for the differences were partial $\eta^2 = 0.162$ and partial $\eta^2 = 0.316$ for the Sydney and Toronto cohorts, respectively, which are considered large effect sizes. Therefore, the only potential concern with obtaining a significant result from a small sample size would be selection bias, which has been mitigated by the investigation of 2 independent cohorts, recruited from 2 different countries, by a different set of clinicians. Third, the TAC is not discernible 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 TAC from cortical bone (both low signals ie, black/dark). To address this limitation, we limited our characterization of the TAC 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 possible that our segmentation underestimated the TAC volumes in both groups and both cohorts. Given the consistency of this underestimation in both groups and both cohorts, we believe this minimally affected the group differences in TAC:muscleR.

The MRI data from the 2 different cohorts were acquired with 2 different scanners, using different imaging parameters. The slightly larger voxel sizes of the Toronto studies may have resulted in increased voxel selection during segmentation. Given the independent nature of the 2 cohorts, we used the Sydney cohort to determine whether there were differences in the TAC:muscleR and the Toronto cohort as an independent replication. Notably, each cohort was compared to controls collected on the same scanner. We found that bilateral TACs were significantly smaller in both cohorts. Yet, given the cross-sectional design of this study, whether the temporalis TAC:muscle abnormalities preexist the onset of mTMD or are a consequence of the disease remains an open question. Therefore, future longitudinal human and/or animal studies are required to disentangle these competing hypotheses.

5. Conclusions

We show in 2 independent cohorts, that patients with chronic mTMD have smaller temporalis TAC volume, and smaller temporalis TAC:muscleR. Future work is required to determine the mechanism and functional significance of reduced temporalis TAC volume in mTMD, and to determine whether such abnormalities exist in other masticatory muscles.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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