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Original Research Article

Foci of Segmentally Contracted Sarcomeres in Trapezius Muscle Biopsy Specimens in Myalgic and Nonmyalgic Human Subjects: Preliminary Results

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Abstract

Objective. The myofascial trigger point hypothesis postulates that there are small foci of contracted sarcomeres in resting skeletal muscle. Only one example, in canine muscle, has been published previously. This study evaluated human muscle biopsies for foci of contracted sarcomeres. **Setting**. The Departments of Rehabilitation Sciences and Physiotherapy at Ghent University, Ghent, Belgium. **Subjects**. Biopsies from 28 women with or without trapezius myalgia were evaluated, 14 in each group. **Methods**. Muscle biopsies were obtained from regions of taut bands in the trapezius muscle and processed for light and electron microscopy and for histochemical analysis. Examination of the biopsies was blinded as to group. **Results**. A small number of foci of segmentally contracted sarcomeres were identified. One fusiform segmental locus involved the entire muscle fiber in tissue from a myalgic subject. Several transition zones from normal to contracted sarcomeres were found in both myalgic and nonmyalgic subjects. The distance between Z-lines in contracted sarcomeres was about 25–45% of the same distance in normal sarcomeres. Z-lines were disrupted and smeared in the contracted sarcomeres. **Conclusions**. A small number of foci of segmentally contracted sarcomeres were found in relaxed trapezius muscle in human subjects, a confirmation of the only other example of spontaneous segmental contraction of sarcomeres (in a canine muscle specimen), consistent with the hypothesis of trigger point formation and with the presence of trigger point end plate noise.

Key Words: Myofascial Trigger Points; Segmental Sarcomere Contraction; Taut Bands; Muscle Biopsy; Trigger Point Hypothesis

Introduction

Myofascial pain syndrome (MPS) is a common cause of both localized and referred pain. The myofascial trigger point (MTP), a small, hard, and tender region in a taut band (TB) of muscle fibers, is considered to be the source of the local and referred pain that characterizes the syndrome. The TB and the MTP persist even in resting muscle. The etiology of the MTP has not been completely elucidated. An increasing accumulation of evidence supports a trigger point hypothesis that postulates that the tender, hard nodule in the TB is associated with multiple foci of segmentally contracted sarcomeres within muscle fibers [1,2]. Simons supported this hypothesis with a single photomicrograph of what he called a "contraction knot" from a canine muscle TB [3]. The image was taken from a study of palpable bands in canine muscle and has never been confirmed or reproduced by a similar finding in either humans or other mammals. However, an oblique, hyperalgesic muscle ipsilateral to the insertion of an artificial stone implanted in the ureter of a rat was evaluated morphologically in a single animal specimen and found to have shortened sarcomeres, suggesting to the authors that the muscle contraction induced by the

ureteral stone was contributory to the hypersensitivity of muscle [4]. There was no further description of the muscle change, and no figures of tissue with shortened sarcomeres were shown.

The TB and the MTP persist long after an acute causative event has resolved, or can develop due to a recurrent cause such as repetitive muscle contraction. The MTP hypothesis postulates that prolonged or repetitive muscle contraction leads to localized, higher, intramuscular pressures that compress capillaries or small arterioles, causing localized muscle ischemia and hypoxia [5]. The resulting increase in proton concentration (acidification) is associated with a rise in the concentration of a number of nociceptive-related neurotransmitters and cytokines and a decrease in adenosine triphosphate (ATP) concentration [6]. Decreased ATP levels can impair reuptake of Ca^{2+} from the muscle cell cytosol, thereby maintaining actin-myosin cross-bridging and muscle contraction [7,8]. Furthermore, lowered ATP levels induce increased spontaneous release of acetylcholine (ACh) from the motor nerve terminal, further promoting segmental muscle contraction, capillary compression, ischemia, and hypoxia [9]. Thus, the MTP becomes self-sustaining and can become chronic. Localized muscle sarcomere contraction is an essential feature of this hypothesis. Therefore, evaluating its presence in myalgic muscle could provide important information regarding the nature of the MTP.

The "contraction knot" has been suggested to be the result of increased ACh at the neuromuscular junction (NMJ). Low-amplitude, high-frequency discharges, similar to miniature end plate potentials (MEPPs), and higher-amplitude, lower-frequency end plate spikes (EPS) make up MTP end plate noise (EPN), the characteristic electromyographic (EMG) MTP signature [10]. EPN is present at the MTP when the muscle is at rest and when non-MTP muscle is almost completely electrically silent. This finding suggests that ACh is pathologically released or leaked to an excessive degree from the motor nerve terminal of the NMJ at the MTP in a nonquantal, spontaneous fashion, rather than in response to efferent, nerve-evoked activity. The EMG data are consistent with an accumulation of ACh at the motor end plate. End plate noise is thought to represent a phenomenon like MEPP and EPS, but at a greatly increased frequency. This could be the result of an abnormal increase of ACh released from the motor nerve terminal in resting muscle, as suggested by the effect of suppression of EPN and EPS by the alpha-adrenergic inhibitor phentolomine, which indicates a presynaptic origin of EPN [11]. It might also be the result of inhibition of acetylcholinesterase (AChE), an increase in acetylcholine receptors in the end plate region, or a combination of these factors [12].

There have been at least two attempts to create a "contraction knot" in skeletal muscle by inhibiting AChE reported in the literature. Mense et al. [13] used diisopropylfluorophosphate to inhibit AChE, but produced highly contracted disc-like foci randomly distributed along the muscle fiber, and contrary to their expectations, not in relation to NMJ, and not resembling Simons and Stolov's fusiform "contraction knot." Another group using neostigmine in a rat model produced foci of hypercontracted sarcomeres that expressed increased MEPP activity [14]. They also showed that the hypercontracted foci were in close proximity to the NMJ.

The present study addresses the problem that Simons' "contraction knot" has never been reproduced by examining muscle tissue obtained by needle biopsy of the trapezius muscle in myalgic and healthy control subjects. The biopsy material used in this study was originally obtained to examine myalgic muscle morphology, looking at fiber type proportion, fiber size, internal nuclei, muscle fiber irregularity, mitochondrial morphology, and lipid droplets [15]. However, sarcomere contraction was not assessed in the original study. Furthermore, as, the study was designed to examine muscle morphology, the biopsies were obtained from the MTP, TBs, or the TB region. Awareness of the availability of this biopsy material led the senior author (RDG) to propose looking at the tissue specifically for the purpose of determining whether focal regions of contracted sarcomeres were present in this muscle biopsy tissue and, if so, whether they would be more likely to be found in myalgic muscle than in nonpainful muscle. It is very unusual to have the opportunity to examine human muscle biopsy material taken not only from myalgic muscle, but also from the MTP, TB, or from the regions of the MTP or TB in the muscle. The objective of the present study, therefore, was to test the hypothesis that foci of contracted sarcomeres exist in human skeletal muscle and that they could be associated with the MTP in muscle biopsies taken from human myalgic and nonmyalgic trapezius muscle.

Methods

Subjects were female office workers with work-related neck pain who were recruited by local advertising in workplaces with predominantly computer-based office tasks. Participants had to have performed computer-related work for at least 20 hours weekly over the course of the previous year. All subjects were between 20 and 50 years of age and were women in order to avoid possible effects of gender on muscle morphology in the original study. Inclusion criteria included neck and shoulder pain that started after, or was aggravated by, computer work for >30 days during the previous year and for at least one day a week. The numerical pain rating score (NPR) had to be at least 2 on a Likert scale of 0 = no pain and 10 = the worst possible pain. Pain could be bilateral or not, but had to be present on the hand-dominant side. Healthy control subjects could not have had neck or shoulder region pain for more than eight days in the preceding year, and never of intensity >2 on an NPR scale of 0–10. Exclusion criteria for both groups

included 1) pregnancy, 2) body mass index >30 kg/m^2, 3) traumatic injury or surgery to the neck or shoulder region, 4) medical illness that could confound the diagnosis of trapezius myalgia such as fibromyalgia or cervical radiculopathy, 5) a history of possible interference with normal coagulation that could be a contra-indication for needle biopsy, including medications that affect coagulation. For a more complete description of the subject population, including population demographics, see De Meulemeester et al. [15].

Subjects who met the screening criteria were examined for trapezius myalgia by a physiotherapist (KDM) who at the time of this analysis was a PhD candidate in the Department of Rehabilitation Sciences and Physiotherapy at Ghent University, Ghent, Belgium. Trapezius myalgia was diagnosed if the subject had palpable tightness of the upper trapezius muscle and tenderness to palpation of the muscle. In addition, relevant MTPs were identified by palpation if there were 1) a palpable taut band, 2) exquisite, localized tenderness within the palpable TB, 3) subject recognition that the pain elicited by palpation resembled their usual pain in whole or in part, and/or 4) palpation elicited referred pain. The subjects were selected, therefore, based on the screening questionnaire to determine whether they qualified as having trapezius myalgia. All subjects were then examined for TBs or MTPs. All subjects were instructed not to take analgesics or muscle relaxants 24 or 72 hours, respectively, before the day of the muscle biopsy.

Patients were assigned to the myalgic group or the control group based on their answers to the online screening questionnaire. That is, if the subjects met the criteria for no shoulder pain more than eight days of the year and did not have a pain intensity of $\geq$2 on the NPS, they were assigned to the control group, regardless of palpatory findings. All subjects were examined for TBs and MTPs on the day of the biopsy. The location of the MTP in the myalgic subjects and the location of the TB region in the nonmyalgic control subjects was marked on the skin at the midpoint of the upper trapezius muscle between the C7 spinous process and the lateral edge of the acromion, which is a common region for the presence of MTPs in the upper trapezius muscle. Ultrasound of the muscle was used to assess the thickness of the muscle and to identify blood vessels in the biopsy field. Local anesthetic (lidocaine 1%) was injected subcutaneously five minutes before the biopsy was performed. Anesthetic was not injected into the muscle. An incision of 2–3 mm was made over the biopsy site. A muscle biopsy (Tru-Cut) was taken by means of a semi-automatic biopsy gun with a 12-gauge needle (Bard Benelux, Olen, Belgium) under ultrasound guidance (Ultrasonography Pro Sound SSD-5000, ALOKA Xo, Ltd. Tokyo, Japan; probe UST-5545, frequency 5–13 MHz). The biopsy needle was directed toward the place in the muscle under the mark on the skin, either an MTP region or a TB region. Each subject was seated and encouraged to relax the shoulders throughout the entire process.

Each biopsy sample was divided in two. One piece was immediately placed in a solution of 4% paraformaldehyde and 5% glutaraldehyde, in 0.1-M cacodylate buffer, and set aside for electron microscopy. Another piece was mounted with Tissue-Tek (OCT compound), frozen in isopentane precooled with liquid nitrogen, and stored at −80°C until processed for other study purposes. All biopsy samples were numbered by an independent, blinded researcher and were therefore evaluated blinded to group assignment. Hence, the examination of the samples for this study was made without knowledge of whether a sample was from a myalgic subject or a healthy control subject.

Electron Microscopy

The fixed muscle sample was cut into 1–2-mm pieces and stained with osmium tetroxide. The tissue was dehydrated with ethanol and propylene oxide. The samples were then placed in BEEM embedding capsules, to which Epon, an embedding medium, was added. The tissue was hardened overnight in an oven at 60°C and then cut into longitudinal sections of 4 μm with a glass microtome system (Ultramicrotomy System, Pyramitone, LKB, Stockholm-Bromma, Sweden). A tissue-orienting staining was performed with toluidine blue, and sections were cut at 90 nm and then 70 nm with a diamond microtome (Reichert Supernova, Leica). The cut sections were stained with uranylacetate and lead nitrate. The sections were then covered with pulverized carbon powder. These procedures were performed by a skilled electron microscope technician in the electron microscopy laboratory of the Pathology Department of Ghent University Hospital, Ghent, Belgium.

Sample Analysis

All samples were evaluated by the first and last authors who were blinded to the group allocation, myalgic or normal, healthy control subjects. Toluidine blue–stained tissue samples were first examined in their entirety for regions of contracted sarcomeres at 40–100× magnification. Those samples showing some degree of contracted sarcomeres were then further evaluated by the electron microscope (EM) at magnifications of 3,000× and 12,000×. Each EM sample was again examined in its entirety for contracted sarcomeres. However, because the tissue that was prepared for EM examination was taken from the larger block that was stained with toluidine blue for light microscopy, some areas of interest seen in the light microscope at lower magnification were not available for EM examination.

Ten randomly selected sarcomeres from contracted regions were compared with adjacent noncontracted regions and were measured using image J 1.50i to gain a general idea of the degree of contraction of the

sarcomeres in the regions of focally contracted sarcomeres. In this manner, the distance between Z-lines in the sarcomere was measured in a sampling of contracted sarcomeres and in nearby, noncontracted sarcomeres for comparison in the same muscle fiber.

This study conformed to all STROBE guidelines and reports the required information accordingly (see the Checklist, Supplemental Digital Content 1, http://links.lww.com/PHM/A661, in De Meulemeester et al. [15]). All subjects gave written informed consent. The study was approved by the Local Ethics Committee of Ghent University Hospital through an amendment to the original approval given for the study reported by De Meulemeester et al. [15].

Results

Subjects

The screening questionnaire was completed by 111 women (Table 1). Thirty-two women met the study criteria and were enrolled in the original study [15]. Myalgic subjects had a median NPR score (interquartile range) of 5 (2–7). The healthy controls were pain-free. Taut bands were found by palpation in the trapezius muscles of all subjects, either bilaterally or on the dominant hand side, both in the myalgic group and in the healthy control group. The difference between the myalgic group and the controls was that the TBs were symptomatic with recognizable pain upon palpation in the myalgic group. Active MTPs that were spontaneously painful were found in the upper trapezius muscle at the midpoint of the muscle between the C7 spinous process and the lateral edge of the acromion in each of the myalgic subjects. No MTPs were found at the corresponding site in nonmyalgic, healthy control subjects. Latent trigger points that were painful only upon stimulation were not recorded in the original study.

Muscle Tissue Examination Findings

Muscle samples were available for evaluation from 15 myalgic subjects and 14 healthy control subjects. In one sample (subject #9, myalgic) the toluidine blue stain had faded to the degree that the sample could not be evaluated and no more material was available for analysis. There was no tissue available for examination from subjects #10 (myalgic) or #32 (control). One locus of highly contracted sarcomeres that was present through the full diameter of the fiber was found in the tissue from subject #20 (Figure 1A), a subject who met the study criteria for trapezius myalgia and who was identified as having a relevant MTP in the upper trapezius muscle. The locus contained about 60 rows of sarcomeres. The site of contraction had an increased cross-fiber width compared with the fiber width on either side of it; that is to say, the contraction site was fusiform or nodular. Three additional foci of less highly contracted, but still definite, examples of segmental (not generalized) sarcomere contraction were also found, one from the same subject #20 and one from subject #4 (Figure 1, B and C, respectively), also a subject with trapezius myalgia. A sample from subject #2 (healthy control) also showed a transition from normal-length sarcomeres to contracted sarcomeres. A fourth tissue sample, from subject #31 (healthy control group), showed several areas of focal segmental contraction at the edge of muscle fibers that did not extend across the entire width of the fiber, as they did in the samples from myalgic subjects #20 and #4 (Figure 1D). In addition, there was some relatively minor variation in sarcomere length affecting up to 4 or 5 rows, of limited extent, most evident at the edge of the fiber.

The length of the contracted sarcomeres, that is, the distance between Z lines as analyzed on EM, was measured in 10 different sites, in some cases more than one site from one subject, and ranged from about 25% to 45% of the normal sarcomere length, when comparing the distance between Z lines in the contracted segments with the adjacent, noncontracted, portions of the same muscle fiber. The region of the "contraction knot" in specimen #20 was only available on the toluidine blue–stained tissue and was not found on the EM sections because of the way the tissue was originally selected and cut for EM examination. The length of the contracted sarcomeres in the contracted region of the "contraction knot" appeared to be about 45% of the length of normal sarcomeres or contracted by a little more than half, based on the light microscopy examination. The Z lines in the most contracted sarcomeres lost their sharp definition and appeared smeared or smudged (Figure 2A and B).

No structure that suggested an NMJ could be identified in close proximity to an area of segmental sarcomere contraction in this small sample. However, there was no attempt to process the tissue in a way that might identify an NMJ, as this was not a goal of the original study.

Discussion

This is the first report of segmentally contracted sarcomeres in skeletal muscle in MTP regions or TB regions since the report by Simons and Stolov [3] over 50 years ago. We hypothesized that foci of segmentally contracted sarcomeres would be found in the region of the MTP or (less likely) in the region of the TB, and indeed, some were found there. However, we could not be certain if the biopsies from the myalgic subjects actually came from an MTP or from a TB. The number of specimens with segmental sarcomere contraction was small, and only one example was found that showed the complete segment looking like a "contraction knot." The uncertainty of the actual site from which tissue was obtained makes it difficult to tell if the small number of specimens with segmental sarcomere contraction means that this phenomenon is uncommon in MTPs and in TBs, or simply that the biopsies were not in sites with many such

Table 1. Myalgia, trigger point, and taut band characteristics of subjects and histology of biopsies

Subject	Myalgic	Normal Control (No Shoulder Pain)	Taut Band Present	Myofascial Trigger Point Present: X	Toluidine Blue Light Microscope, 40–100× Magnification	Electron Microscope, 3,000–12,000× Magnification	Comment
1		X	X		0		
2		X	X		Segmental contraction at one side of fiber only	Segmental sarcomere contraction	Sarcomere contraction did not extend across entire fiber
3	X		X	X	0	0	
4	X		X	X	Segmental contraction present	Segmental contraction present with region of transition from full to contracted sarcomeres	
5	X		X	X	0	0	
6		X	X		2 small areas of contraction only at edge of fiber		Thought to be artifact
7	X		X	X	0	0	
8		X	X		0		
9	X		X	X	–		Stain faded, could not be analyzed
10	X		X	X	–		No tissue available
11	X		X	X	0	0	
12		X	X		0	0	
13	X		X	X	0	0	
14	X		X	X	0	0	
15		X	X		0	0	
16	X		X	X	0	0	
17	X		X	X	0	0	
18	X		X	X	0	0	
19		X	X		Mild variation in sarcomere size but no contracted sarcomeres	0	
20	X		X	X	Segmental contraction across full fiber width	Areas of transition from normal to contracted sarcomeres; smeared Z-lines	The full fiber thickness segmental sarcomere contraction tissue was not available for EM examination but other tissue from this subject was available
21		X	X		0	0	
22	X		X	X	0	0	
23		X	X		0	0	
24	X		X	X	0	0	
25		X	X		0	0	
26	X		X	X	0	0	
27	X		X	X	0	0	
28		X	X		0	0	
29		X	X		1 small region of sarcomere contraction	1 section of 4–6 rows of contracted sarcomeres	
30		X	X		0	0	
31		X	X		Sarcomere contraction at the edge of the tissue	Limited contraction at edge of fiber	
32					–		No tissue or no subject
33		X	X		0	0	

foci. In addition, this is the first report of Z band dissolution in segmentally contracted sarcomeres in or near MTPs or TBs. The Integrated Hypothesis of the Trigger Point postulates that "contraction knots" should be found in the MTP, but neither the original report by Simons and Stolov [3] nor our specimens can be said with certainty to locate this at an MTP. Highly localized regions of intense sarcomere contraction are a central feature of the "Integrated Trigger Point Hypothesis" [16]. The experimental support for this largely rests on

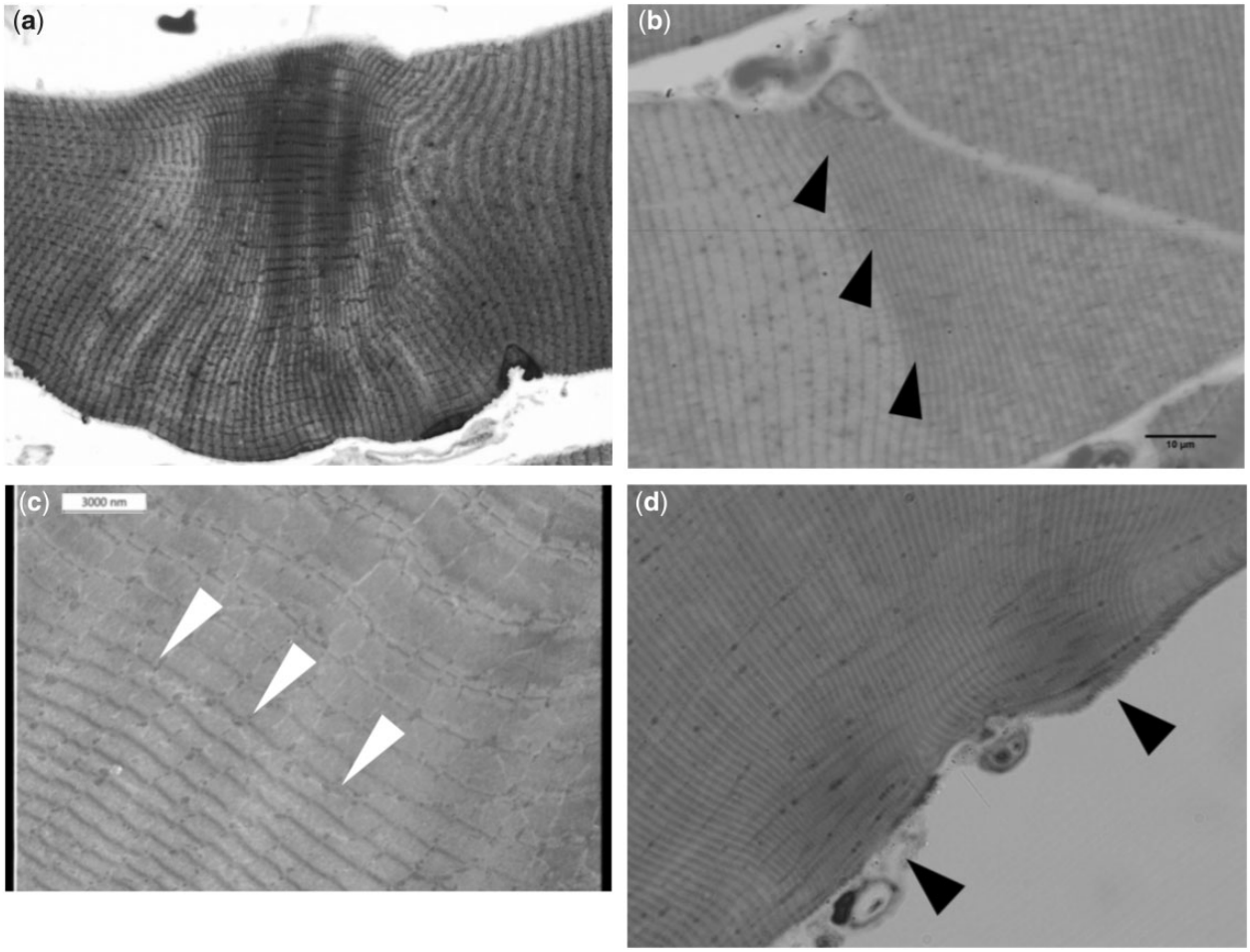

Figure 1. Photomicrographs of human skeletal muscle from biopsies of palpable bands of muscle in subjects with the muscle at rest. A) Fusiform enlargement of a muscle fiber at a region of segmental sarcomere contraction taken from a myalgic subject (subject #20), at 100X magnification, showing the contracted sarcomeres, and the increase in the cross-section diameter of the fiber. B) Transition zone between normal and contracted sarcomeres (black arrowheads) from myalgic subject #20 (3000X magnification), showing the transition from sarcomeres with greater inter-Z-line distance to those with shorter inter-Z-line distances consistent with shortening of the sarcomeres. C) Transition zone between normal and contracted sarcomeres (white arrow heads) in myalgic subject #4 (3000X magnification),showing the transition from sarcomeres with greater inter-Z-line distance to those with shorter inter-Z-line distances consistent with shortening of the sarcomeres. D) Two regions of contracted sarcomeres that are limited to the edge of the muscle fiber, not extending through the full diameter of the fiber, in a non-myalgic healthy control subject (magnification 3000X).

the electromyographic data that are consistent with localized regions of muscle cell membrane depolarization, resulting in high-frequency MEPP and EPS in resting muscle. Thus, the finding of segmentally contracted sarcomeres takes on importance only in the context of data, like the electromyographic data. At present, there is no evidence that they are found in significantly greater numbers in the MTP regions compared with non-MTP regions, a concern our study does not address.

There are very few such examples of segmentally contracted sarcomeres reported in the literature. In fact, to our knowledge, the Simons and Stolov [3] paper, a study of segmental muscle fiber lesions after repetitive eccentric contractions [17], and our report are the only such reports of segmental sarcomere contraction in in vivo skeletal muscle, as opposed to myocardial or smooth muscle. A report by Giamberardino et al. [4] citing sarcomere contraction in hyperalgesic body wall muscle ipsilateral to artificial ureteral lithasis did not mention whether the contraction was segmental, but merely stated that contracted sarcomeres were found in the affected muscle in the one animal that was studied morphologically. Mense and Gerwin showed one photomicrograph of an electron microscopic section taken from a human biopsy specimen from a patient with an MTP, showing a transition zone from normal sarcomeres to shortened sarcomeres, but that did not include an entire contraction node [18]. This was part of an unfinished study that was never completed and never published.

Only a few examples of segmental sarcomere contraction were present in the tissue specimens available in this study, and only one complete fusiform example.

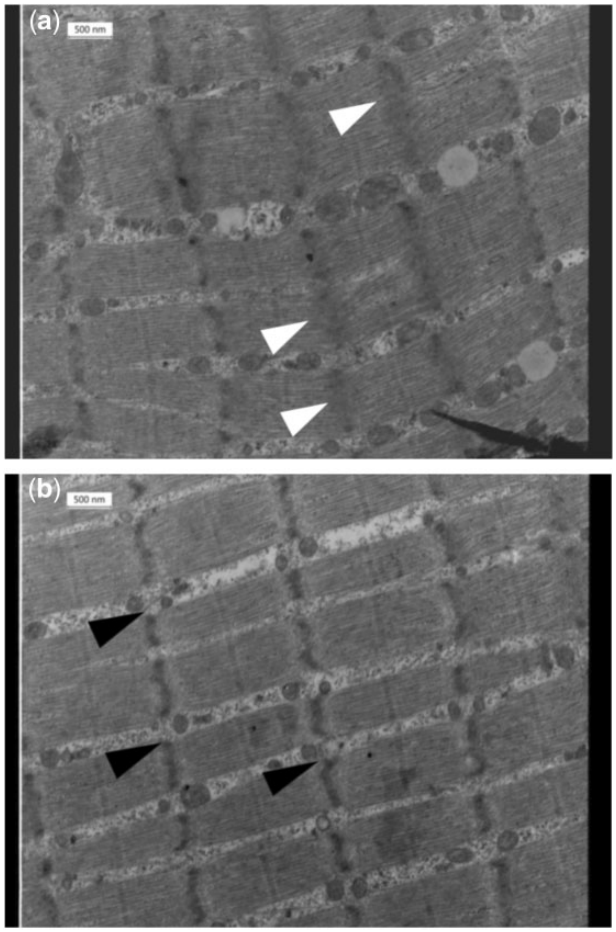

Figure 2. A) Electron microscopic image of muscle sarcomeres taken from a palpable band in trapezius muscle from myalgic subject #20, showing disrupted and smeared Z-lines (12000X magnification. B) Electron microscopic image of non-contracted sarcomeres taken from a palpable band in the same myalgic subject #20 showing normal Z-lines for comparison.

Segmental sarcomere contraction was found in two of the 15 myalgic subjects with available biopsy material. Two additional samples from healthy controls showed small regions of sarcomere contraction at the edge of the sample. The other biopsy specimens in our study showed rather regular spacing of sarcomeres with only slight variation in sarcomere length and no regions of segmentally contracted sarcomeres. As we could not verify that the biopsies actually came from an MTP or a TB, this cannot be considered a quantitative study of the incidence of foci of contracted sarcomeres in either MTPs or TBs.

Myofascial trigger points were found in each of the myalgic subjects, but in none of the healthy control subjects. The intention was to biopsy the MTP when it was present, or from a TB, which was present in all subjects. We could not confirm the actual site of the biopsy, but we do not consider tissue taken from the TB or near it to be a surrogate of, or to represent, an MTP. Electromyographic studies of the local twitch response in rodents show that as the distance from the trigger zone in the TB increases, the amplitude of the EMG response decreases and finally disappears [19]. It is likely, therefore, that the trigger zone is highly localized in the taut band. Moreover, because the tissue was obtained for purposes different from this study, the number of specimens required to show a credible difference between normal and myalgic tissue was not determined. It should be no surprise, then, that the biopsies might show few or no features postulated to be characteristic of the MTP.

It has been suggested that if contraction knots do exist in MTPs, then they should be in close proximity to the NMJ [14]. Identification of the NMJ was not an aim of either this study or the original study from which the biopsy tissue was obtained, and no attempt was made to identify the elements of the NMJ. Consequently, we cannot say anything about such a relationship.

We hypothesize that regions of sarcomere contraction are spatially related to MTPs, although neither this study nor Simons and Stolov's microphotograph can prove this. It is possible that foci of contracted sarcomeres occur randomly throughout resting muscle normally and are unrelated to trigger points or to muscle pain. Our findings, however, do not address that, although the vast majority of our small sample of specimens did not show any foci of contracted sarcomeres. It is also possible that foci of contracted sarcomeres occur for reasons we have not considered that are unrelated to the formation of MTPs and to the process of nociception. Furthermore, it is possible that foci of contracted sarcomeres occur as the result of the way the samples are processed, and are therefore artifacts of processing. Margalef et al. [14] stated that using the fixative paraformaldehyde could cause contracted muscle tissue and a blurry visualization of the contraction knots. All of our samples were fixed in glutaraldehyde and paraformaldehyde. However, our biopsy specimens retained good ultrastructure definition. Additionally, 85% of the samples did not show focal sarcomere contraction and maintained good light and EM morphology with no more than minimal variation in sarcomere length. Therefore, it is possible, and we believe more likely than not, that focal sarcomere contraction is not an artifact of tissue fixation and processing. Hudson et al [20] also produced segmental sarcomere contraction closely related to the NMJ using low dose neostigmine.

Attempts have been made to find examples of focal sarcomere contraction in cadaver muscle tissue because of the difficulties in obtaining skeletal muscle biopsies from MTP sites in human subjects [21]. Examples of sarcomere contraction in cadaver material are difficult to assess, because the ATP that is required to remove ionized Ca^{2+} from the cytosol is no longer available and therefore actin-myosin cross-bridging persists.

Taut bands persist in resting muscle, the characteristic the EMG signature of the trigger point, EPN, persists at rest [10,19,22], and ultrasound images show dense bands of muscle at rest [23].

Smearing of the Z bands in the segmentally contracted sarcomeres is of potential interest in that titin attaches to

the Z band and connects it to the M band and also connects the Z band to the thick filament myosin [24,25]. Dysfunction of titin related to dissolution of the Z bands could be related to abnormal static tension and the development of the TB, but we have no information about the structure of titin in the specimens reported from this study and cannot make any further comment about this possibility.

Trigger points are found in TBs, and not elsewhere. TBs in this study were found in all subjects, whether they had myalgia or not. The epidemiology of the TB was not a subject of either the original study or this analysis, but the observation is of interest, as there are no studies that specifically focus on the prevalence or incidence of TBs in myalgic and nonmyalgic subjects.

The strengths of this study are that all subjects were examined for TBs and MTPs by one experienced physiotherapist, that all biopsies were done by one clinician, that tissue was obtained by in vivo biopsy from humans, that the biopsy needle was directed toward the MTP, TB, or the TB region, that the subjects were instructed to relax their shoulders during the biopsy procedure in order to promote the biopsy of resting muscle, and that material was available for both light and electron microscopic examination. Furthermore, the clinicians examining the specimens were blinded to the subject group, myalgic or healthy, normal controls.

There are a number of limitations to this study. In the first place, the tissue was obtained for purposes other than the purpose of this study. Therefore, the tissue was handled in a manner that did not allow a more complete examination for sarcomere contraction, for nerve fibers, or for the structures of the NMJ. The process of procuring and processing the tissue could also have altered the preparations and perhaps have created artifact. The biopsies taken in our study were taken with the subjects being told to put the trapezius muscle at rest, but some inadvertent muscle contraction at the time of biopsy cannot be excluded. Furthermore, tissue from needle biopsies tends to be fragmented or irregular, potentially giving rise to artifact. Larger muscle fragments would be more suitable for analysis than smaller fragments, but would require more invasive muscle biopsy procedures.

Though an attempt was made to biopsy muscle from an MTP, the TB, or the TB region, the exact location of the biopsy could not be confirmed. Therefore, we cannot say that the single fusiform region of sarcomere contraction is relevant to an MTP, or for that matter, even to a TB. The issue that foci of contracted sarcomeres are related to MTPs and are not artifactual or due to other causes may not be settled until biopsy material is taken specifically from the MTP site itself, rather than from the MTP region or from palpable bands, and compared with non–trigger point muscle tissue. Identifying the NMJ and ACh receptors in biopsy material would help determine if localized sarcomere contraction is spatially related to the NMJ and if an increase in ACh receptors may play a role in localized, subthreshold release of Ca^{2+} in the muscle cell.

Conclusions

The objective of this study—to test the Integrated Hypothesis of the Trigger Point, which postulates that segmentally contracted sarcomeres, or "contraction knots," are a feature of the MTP region of TBs—was met with the finding of one locus of a fusiform, segmentally contracted series of sarcomeres and several other areas of segmental sarcomere contraction. The paucity of the findings strongly suggests that a study of tissue taken specifically from the MTP itself is needed to settle this question. Nevertheless, this is the first time that the finding of in vivo segmentally contracted sarcomeres in skeletal muscle has been reproduced since Simons and Stolov's first report [3]. This is also the first time that a fusiform locus of contracted sarcomeres has been clearly shown in human muscle and the first time that smeared Z-lines have been reported in segmentally contracted sarcomeres taken from tissue that could be from either the MTP region or the TB region of the skeletal muscle. The results of the present study must be interpreted with caution, as we cannot exclude the possibility that our finding, or, for that matter, the finding of Simons and Stolov, represents changes that are either artifactual or that are unrelated to MTPs. Finally, this is the first time that TBs have been reported to be found in all subjects, whether myalgic or nonmyalgic, regardless of the presence of MTPs and regardless of the presence of pain.

References

1. Gerwin RD. Trigger Point neurophysiology. In: Donnelly JM, Fernández de las Peñas C, Finnegan M, Travell JF, eds. Simons & Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual. 3rd ed. Philadelphia: Walters Kluwer; 2019:29–43.
2. Shah JP, Thaker N, Heimur J, Aredo JV, Sikdar S, Gerber L. Myofascial trigger points then and now: A historical and scientific perspective. PM & R 2015;7(7):746–61.
3. Simons DG, Stolov WC. Microscopic features and transient contraction of palpable bands in canine muscle. Am J Phys Med 1976;55(2):65–88.
4. Giamberardino MA, Affaitati G, Lerza R, et al. Evaluation of indices of skeletal muscle contraction in areas of referred hyperalgesia from an artificial ureteric stone in rats. Neurosci Lett 2003; 338(3):213–6.
5. Bron C, Dommerholt JD. Etiology of myofascial trigger points. Curr Pain Headache Rep 2012;16(5):439–44.
6. Shah JP, Phillips TM, Danoff JV, Gerber LH. An in vivo microanalytic technique for measuring the local biochemical milieu of human skeletal muscle. J Appl Physiol 2005;99(5):1977–84.
7. Brini M, Carafoli E. Calcium pumps in health and disease. Physiol Rev 2009;89(4):1341–78.
8. Carafoli E. Calcium-mediated cellular signals: A story of failure. Trends Biochem Sci 2004;29(7):371–9.
9. Vyskočil F, Malamouzh AI, Nikolsky EE. Non-quantal acetylcholine release at the neuromuscular junction. Physiol Res 2009; 58:763–84.

10. Simons DG, Hong CZ, Simons LS. Endplate potentials are common to mid-fiber myofascial trigger points. Am J Phys Med Rehabil 2002;81(3):212–22.
11. Chen JT, Chen SM, Kuan TS, Chfung KC, Hong CZ. Phentolamine effect on the spontaneous electrical activity of active loci in a myofascial trigger spot of rabbit skeletal muscle. Arch Phys Med Rehabil 1998;79(7):790–4.
12. Gerwin RD, Dommerholt J, Shah J. An expansion of Simons' integrated hypothesis of trigger point formation. Curr Pain Headache Rep 2004;8(6):468–75.
13. Mense S, Simons DG, Hoheisel UL, Quenzer B. Lesions of rat skeletal muscle after local block of acetylcholinesterase and neuromuscular stimulation. J Appl Physiol 2003;94(6):2494–501.
14. Margalef R, Sisquella M, Bosque M, et al. Experimental myofascial trigger point creation in rodents. J Appl Physiol 2019;126 (1):160–9.
15. De Meulemeester K, Calders P, Van Dorpe J, De Pauw R, Petrovic M, Cagnie B. Morphological differences in the upper trapezius muscle between female office workers with and without trapezius myalgia: Facts or fiction? A cross-sectional study. Am J Phys Med Rehabil 2019;98(2):117–24.
16. Simons DG, Travell JG, Simons LS. Myofascial Pain and Dysfunction: The Trigger Point Manual. Baltimore, Williams & Wilkins; 1999:69–78.
17. Fridén J, Lieber R. Segmental muscle fiber lesions after repetitive eccentric contractions. Cell Tissue Res 1998;293:165–71.
18. Mense S, Gerwin RD. Muscle Pain: Diagnosis and Treatment. Berlin: Springer-Verlag; 2010.
19. Hong CA, Torigoe Y. Electrophysiologic characteristics of localized twitch responses in responsive taut bands of rabbit skeletal muscle. J Musculoskelet Pain 1994;2(2):17–43.
20. Hudson CS, Rash JE, Tiedt TN, Albuquerque EX. Neostigmine-induced alterations at the mammalian neuromuscular junction. II. Ultrastructure. J Pharmacol Exp Ther 1978;205(2):340–56.
21. Reitinger A, Radner H, Tilscher H, Hanna M, Windisch A, Feigl W. Morphologic study of trigger points [in German]. Man Med 1996;43:256–62.
22. Fernández-Carnero J, Ge HY, Kimura Y, Fernández-de-Las-Peñas C, Arendt-Nielsen L. Increased spontaneous electrical activity at a latent myofascial trigger point after nociceptive stimulation of another latent trigger point. Clin J Pain 2010;26(2): 138–43.
23. Sikdar S, Shah JP, Gebreab T, et al. Novel applications of ultrasound technology to visualize and characterize myofascial trigger points and surrounding soft tissue. Arch Phys Med Rehabil 2009;90(11):1829–38.
24. Nocella M, Cecchi G, Bagni MA, Colombini B. Force enhancement after stretch in mammalian muscle fiber: No evidence of cross-bridge involvement. Am J Cell Physiol 2014;307(12):C1123–9.
25. Tskhovrebora L, Trinick L. Role of titin in structure and elasticity of the sarcomere. J Biomed Biotechnol 2010;2010: 612482.