ORIGINAL ARTICLE

Biochemicals Associated With Pain and Inflammation are Elevated in Sites Near to and Remote From Active Myofascial Trigger Points

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ABSTRACT. Shah JP, Danoff JV, Desai MJ, Parikh S, Nakamura LY, Phillips TM, Gerber LH. Biochemicals associated with pain and inflammation are elevated in sites near to and remote from active myofascial trigger points. Arch Phys Med Rehabil 2008;89:16-23.

Objectives: To investigate the biochemical milieu of the upper trapezius muscle in subjects with active, latent, or absent myofascial trigger points (MTPs) and to contrast this with that of the noninvolved gastrocnemius muscle.

Design: We used a microanalytic technique, including needle insertions at standardized locations in subjects identified as active (having neck pain and MTP), latent (no neck pain but with MTP), or normal (no neck pain, no MTP). We followed a predetermined sampling schedule; first in the trapezius muscle and then in normal gastrocnemius muscle, to measure pH, bradykinin, substance P, calcitonin gene-related peptide, tumor necrosis factor alpha, interleukin 1β (IL-1β), IL-6, IL-8, serotonin, and norepinephrine, using immunocapillary electrophoresis and capillary electrochromatography. Pressure algometry was obtained. We compared analyte concentrations among groups with 2-way repeated-measures analysis of variance.

Setting: A biomedical research facility.

Participants: Nine healthy volunteer subjects.

Interventions: Not applicable.

Main Outcome Measures: Preselected analyte concentrations.

Results: Within the trapezius muscle, concentrations for all analytes were higher in active subjects than in latent or normal subjects ($P<.002$); pH was lower ($P<.03$). At needle insertion, analyte concentrations in the trapezius for the active group were always higher (pH not different) than concentrations in the gastrocnemius muscle. At all times within the gastrocnemius, the active group had higher concentrations of all analytes than did subjects in the latent and normal groups ($P<.05$); pH was lower ($P<.01$).

Conclusions: We have shown the feasibility of continuous, in vivo recovery of small molecules from soft tissue without harmful effects. Subjects with active MTPs in the trapezius muscle have a biochemical milieu of selected inflammatory mediators, neuropeptides, cytokines, and catecholamines different from subjects with latent or absent MTPs in their trapezius. These concentrations also differ quantitatively from a remote, uninvolved site in the gastrocnemius muscle. The milieu of the gastrocnemius in subjects with active MTPs in the trapezius differs from subjects without active MTPs.

Key Words: Inflammation; Microdialysis; Myofascial pain; Rehabilitation; Trigger points, myofascial.

MYOFASCIAL PAIN SYNDROME (MPS), a common type of nonarticular musculoskeletal pain, is a condition associated with regional pain and muscle tenderness characterized by the presence of hypersensitive nodules, also called myofascial trigger points (MTPs).[1] MPS affects up to 95% of people with chronic pain disorders and is a common finding in patients in specialty pain management centers.[2]

MTPs are easily identified through physical examination as hyperirritable nodules within taut bands of skeletal muscle, the palpation of which can produce a muscle twitch and referred pain.[1,2] Active MTPs are associated with pain, are acutely tender to palpation, and may contribute to general motor dysfunction (stiffness and restricted range of motion). Latent MTPs, which have similar physical findings, are often associated with motor dysfunction and muscle tenderness, but without spontaneous pain. Normal or uninvolved muscle does not contain taut bands or MTPs.[1]

In acute muscle pain, local tenderness is often caused by the peripheral sensitization of local muscle nociceptors.[3] Nociceptive terminals in muscle have a multitude of different receptors in their membranes, including matched receptors for molecules that are released from damaged tissue such as bradykinin, serotonin, protons (H^+), and prostaglandins.[3] Furthermore, continuous activation of nociceptors by these and other endogenous substances can lead to central sensitization of dorsal horn neurons.[3] The continued presence of these inflammatory mediators and other biochemicals may be necessary for persistent pain conditions such as MPS.[3] There has been little documentation, however, of the biochemical differences between the local tissue milieu of normal muscle and muscle with nonpainful or painful MTPs.

Although the physical findings of myofascial pain involve the local muscle tissue, we have observed that standard treatment approaches have been largely empirical and suboptimal. Medications may provide moderate symptom improvement without complete resolution of the symptoms or the elimination of the MTP.[4]

See commentary, p 157.

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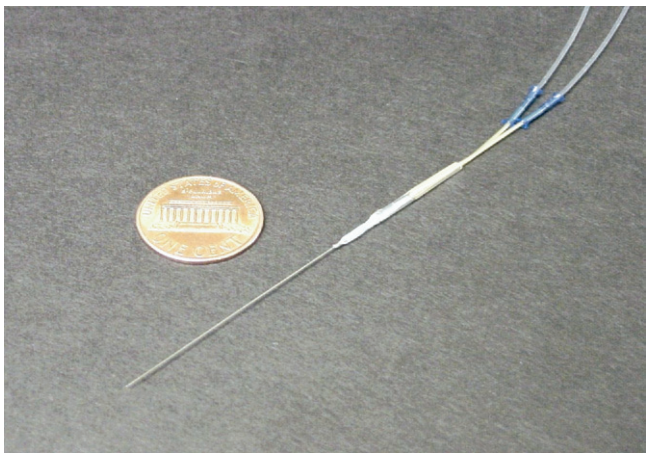

Fig 1. Microdialysis needle.

Eliciting a local twitch response (LTR)* through dry needling of MTPs often has a therapeutic benefit.[5] Mechanisms for this are hypothetical and have not been established. Associations between endogenous substances and muscle pain have been shown, but the pathogenesis of myofascial pain remains unclear. Assaying the local milieu before, during, and after an LTR could potentially disclose changes in bioactive substances that may contribute to pain. This could then lead to the development of treatments that target underlying mechanisms.

In a previous study,[6] we explored the local milieu using a novel microanalytical system, including a minimally invasive 30-gauge needle, that enabled the in vivo collection of small volumes ($\approx 0.5\mu L$) and sub-nanogram sizes ($<75kDa$) of solutes from muscle tissue (fig 1). This system gave us the capability to safely explore and measure the local biochemical milieu of MTPs before, during, and after an LTR.

We found differences in the local biochemical milieu (eg, inflammatory mediators, neuropeptides, catecholamines, cytokines) of subjects with active MTPs, compared with subjects with latent MTPs and those without MTPs, at a standardized location in the upper trapezius muscle.[6] Furthermore, the local milieu does appear to change with the occurrence of an LTR, and these changes can be monitored almost continuously by using short (10–30s) collection intervals. An important, unanswered question is whether the differences in the biochemical milieu between active, latent, or no MTPs pertain to the upper trapezius exclusively, or whether these differences are also present in remote, uninvolved sites.

Our purposes in this study were: (1) to determine the reproducibility of previous findings that showed the unique biochemical milieu of substances associated with pain and inflammation in an active MTP in the upper trapezius muscle, and (2) to compare the similarity of analyte levels sampled from the biochemical milieu in the upper trapezius of subjects with active, latent, and no MTPs with those levels in a remote uninvolved site in the upper medial gastrocnemius muscle.

*Local twitch response refers to the brief local contraction that occurs when a trigger point is stimulated by snapping palpation or needle penetration. Dry needling refers to stimulation of a trigger point, usually with a narrow object such as a blunt needle or empty hypodermic needle.

METHODS

Participants

Subjects were recruited from members of the staff of the clinical center at the National Institutes of Health (NIH). All subjects underwent a thorough musculoskeletal evaluation so as to rule out potential causes of their symptoms other than MTPs in the upper trapezius muscle. Exclusion criteria included a history of the following conditions: fibromyalgia, cervical or lumbar radiculopathy, other pain syndromes, or cancer. Subjects with knee pain, pathology or infection, previous injection treatments, bleeding disorders, and psychologic issues (eg, fear of needles), or who were taking medications or were smokers, were excluded. Subjects who had calf pain or MTPs in the upper medial gastrocnemius were also excluded. None of the subjects had active or latent trigger points in the region of the gastrocnemius in which the needle was inserted. Two clinicians examined all subjects (JPS, MJD). The senior examiner (JPS) is a practicing physician experienced in the evaluation and treatment of musculoskeletal disorders and is an instructor in these techniques.

Based on their history and physical findings in the upper trapezius muscle, 9 subjects were recruited. The 9 were then assigned to 1 of 3 groups: (1) active (MTP present, idiopathic cervical pain of <3mo duration; 3 subjects); (2) latent (MTP present, no cervical pain; 3 subjects); and (3) normal (MTP absent, no cervical pain; 3 subjects). A fourth subject initially recruited for the active group was disqualified. Post hoc review of her medications and clinical history (ie, an unknown cervical radiculopathy) led to the decision that she did not qualify for the active group.

An institutional review board at the NIH approved the study protocol and all subjects signed an informed consent document approved by that board.

Instrumentation

We have previously described our instrumentation.[6] In brief, we used a microdialysis technique to sample the substances of interstitial fluid, which diffused through a semi-permeable membrane at the tip of a 30-gauge stainless steel hypodermic needle.[6,7] Analyte levels in the samples were quantified using immunoaffinity capillary electrophoresis and capillary electrochromatography analysis, 2 techniques known to provide reliable results.[8-10]

Procedures

Subjects completed a visual analog scale (VAS) to document self-reported pain intensity, and underwent pressure algometry[a] at GB-21 in the upper trapezius and LV-7 in the medial gastrocnemius.[11] GB-21 is a standard acupuncture location in the suprascapular region, midway between the tip of the acromion process and below the spinous process of the C7 vertebra. LV-7 is located on the medial side of the leg, inferior to the medial condyle of the tibia, in the upper portion of the medial head of the gastrocnemius muscle. We selected these points to standardize procedures for all subjects. We used pressure algometry to measure local pain (pressure pain threshold [PPT]) bilaterally in all subjects. Algometry has produced valid and reliable data relative to local pain.[11]

Six microdialysis needles were fabricated for this study. They were gas-sterilized between uses. The microdialysis system was calibrated as in the previous study.[6]

For the experimental procedures, subjects were comfortably positioned prone on a standard clinical exam table, with 2 pillows for support and stabilization. The microdialysis needle was then inserted into the upper trapezius muscle at point GB-21 without penetrating the MTP (if present). Muscle penetration was con-

Table 1: Labels and Definitions of Events Used in Repeated-Measures ANOVA

Event Designation	Time (min)	Location	Description
Pre	1:00 2:00	Trapezius	Needle insertion
Peak	5:00 5:20	Trapezius	Needle advancement and LTR
Post	10:00 11:00	Trapezius	Recovery
Calf*	2:00 3:00	Gastrocnemius	Needle insertion

*Calf event times were independent of trapezius event times.

firmed by change in tissue resistance at needle contact with the muscle and by surface electromyography pickup. The needle was kept stationary in situ for 1 minute, after which collection of the sample commenced. Five minutes after insertion, the needle was advanced into the muscle until an LTR was obtained in the active and latent subjects. Again, this was confirmed by surface electromyography. Depth of penetration was estimated to be between 0.5 and 1.0cm. Equivalent needle advancement was used in the normal subjects although an LTR was not observed. The dialysate (physiologic saline) was pumped through the system at a flow rate of 5μL a minute for the entire procedure. Dialysate was sampled according to the following schedule: every minute for the first 4 minutes, then every 20 seconds for 4 minutes (minutes 4–8), then every minute for another 6 minutes (minutes 8–14). Total collection time was 14 minutes. The collection schedule is summarized in appendix 1.

After 14 minutes, the needle in the trapezius muscle was removed and subjects were repositioned in a comfortable supine position with the knees supported by a pillow. A second sterile microdialysis needle was then inserted into the upper medial gastrocnemius muscle at LV-7 and held in this position for 10 minutes, with samples being collected every minute. Depth of penetration was approximately 0.5cm. The needle remained stationary during this procedure because a local twitch response was not desired (and was not expected to occur in normal muscle). A flow rate through the system of 5μL a minute was maintained during the calf procedure.

All samples (22 from the trapezius, 10 from the gastrocnemius for each subject) were analyzed by immunoaffinity capillary electrophoresis, capillary electrochromatography, and micro-pH (a modified microcombination electrode in combination with an Orion model 370[b] pH meter) for pH and 9 different analytes including bradykinin, substance P (SP), calcitonin gene-related peptide (CGRP), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, serotonin (5-HT), and norepinephrine.

Statistical Design and Analysis

We used 2, 1-way analyses of variance (ANOVAs) to compare VAS scores and algometry values among the 3 groups.

Analyte concentrations for all subjects were averaged at each time point. This produced 3 concentration versus time curves for each analyte in the trapezius and 3 curves in the gastrocnemius muscles. Eight of the analytes recovered in this study were also recovered from the trapezius in our previous work,[6] in which we used subjects with inclusion and exclusion criteria similar to those of the current study. These were pH, SP, CGRP, bradykinin, TNF-α, IL-1β, 5-HT, and norepinephrine. Concentration curves of these analytes from the trapezius muscles of subjects in this study were graphically compared with data from our previous study.[6] These data were also compared through 2-way repeated-measures ANOVA using time (repeated variable: early, mid, late) and study (current vs previous).

Data from the previous and current samples were then pooled to evaluate analyte concentrations; we used 2-way repeated-measures ANOVA with time as the repeated variable and group (active, latent, normal) as a nonrepeated variable.

We did a third analysis with the current data to compare analyte concentrations in the trapezius muscle with analyte concentrations in the gastrocnemius muscle. The between-group variable again had 3 levels: active, latent, and normal. The within-group (repeated) variable included 3 collection events for the trapezius and 1 for the gastrocnemius (table 1).

Post hoc testing of significant ANOVA findings was done with protected t tests subject to Bonferroni adjustments of α levels. Data variability has been represented on the figures by the standard error of the mean, which is the dispersion of observations around a sample mean.

RESULTS

Subjects in the active group reported greater pain levels ($P<.001$) on the VAS than did subjects in the latent and normal groups. The active group also had a lower PPT in both the trapezius and gastrocnemius muscles, which would indicate a greater sensitivity to direct pressure. The PPT differences were not significant, however.

In comparing data from subjects previously sampled[6] with the subjects in this study, we found close agreement of biochemical concentrations. Figure 2 shows, as examples, pH and SP. ANOVA indicated that with data from both studies combined, concentrations of all analytes for the active group were higher than concentrations in the latent and normal groups (pH level is lower). There were no statistical differences between analyte concentrations from the previous study and this study.

The averaged data for the remaining biochemical concentrations from subjects in this study are displayed in figures 3

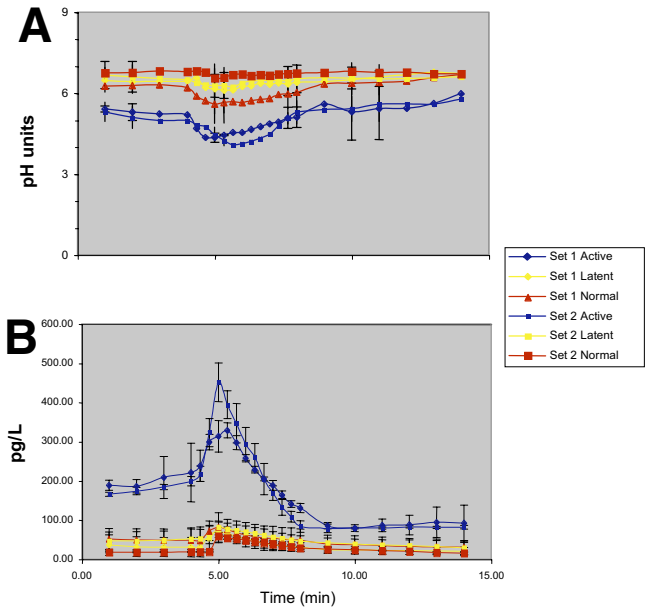

Fig 2. Analyte concentrations in the trapezius combining previous and current data. Collection for (A) pH and (B) SP.

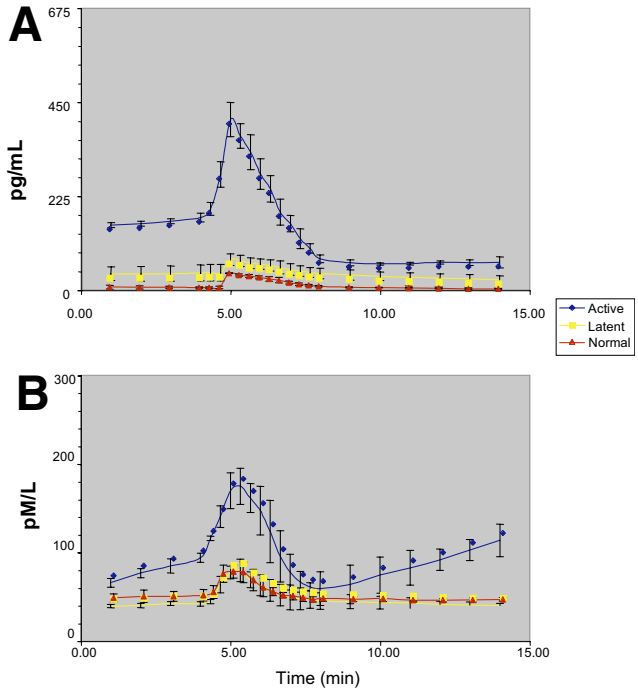

Fig 3. Analyte concentrations in the trapezius for (A) CGRP and (B) bradykinin.

through 6 (trapezius muscle only). Examining the ANOVA main factor group is equivalent to collapsing the time factor (ie, combining all time levels together). This enabled us to compare

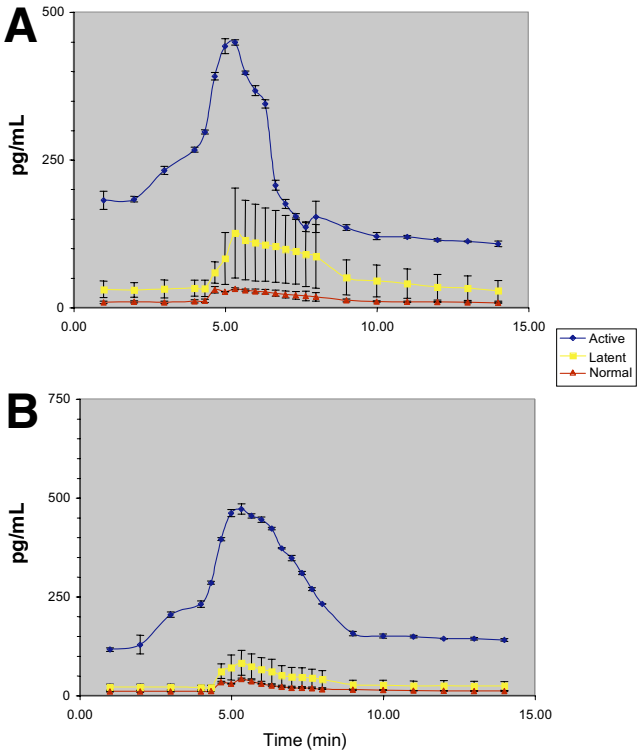

Fig 4. Analyte concentrations in the trapezius for (A) TNF-α and (B) IL-1β.

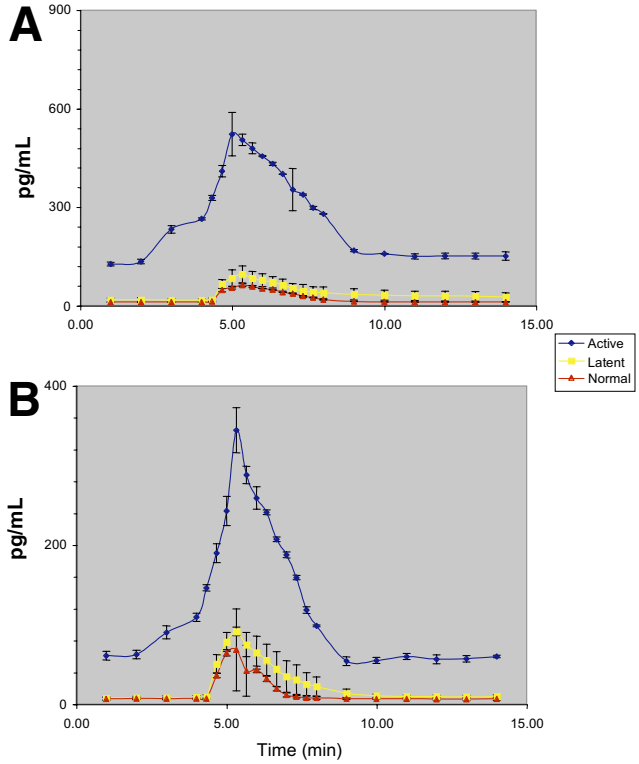

Fig 5. Analyte concentrations in the trapezius for (A) IL-6 and (B) IL-8.

the overall analyte concentrations in the trapezius muscle across the 3 groups. Concentrations for all analytes in the trapezius, except pH, were significantly higher in active than in latent or normal subjects ($P<.002$). In the active group, the recovery (post) amounts of SP and CGRP were significantly lower than the baseline (pre) levels ($P<.02$). For pH, the active group was significantly lower ($P<.03$). There were no overall differences between the latent and normal subjects.

We also found differences in analyte concentrations in the gastrocnemius muscles among the 3 groups. In general, the active group had higher concentrations of all analytes than did those in the latent and normal groups ($P<.05$). In the active group, pH was lower ($P<.01$). Figures 7 through 9 show comparisons of gastrocnemius concentrations with trapezius concentrations. Statistical relationships are summarized in table 2.

We used repeated-measures ANOVA to compare analyte concentrations within the trapezius at specified events (see table 1) with those in the gastrocnemius. We used post hoc testing to identify individual level differences. In all cases, significance was adjusted, using the Bonferroni method, to be at least less than .05. For the active group, analyte concentrations in the gastrocnemius were almost always lower than concentrations in the trapezius. For the latent group, gastrocnemius concentrations were consistently lower than the peak trapezius concentrations, but not necessarily for other time levels. The normal group was similar to the latent group except there were no significant differences in TNF-α and norepinephrine. Table 3 summarizes the comparisons of trapezius and gastrocnemius levels.

DISCUSSION

We have confirmed that biochemicals associated with pain and inflammation are elevated in soft tissue in the vicinity of

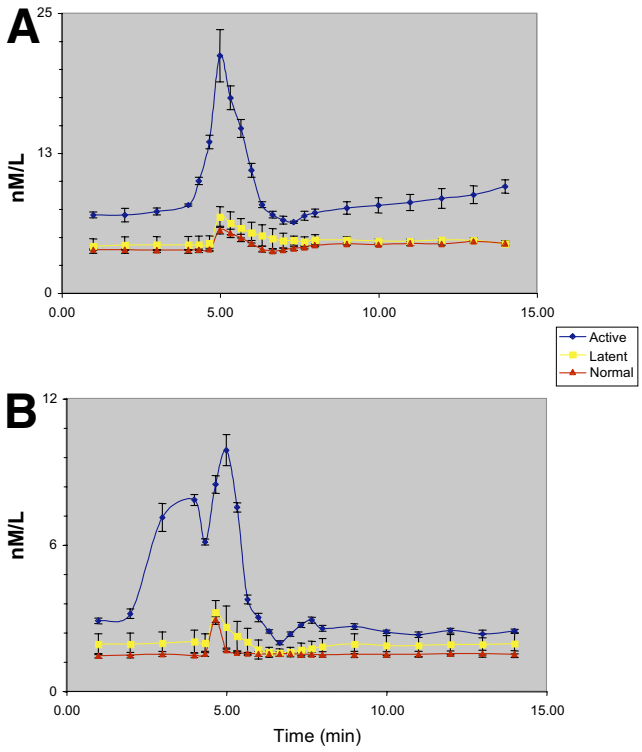

Fig 6. Analyte concentrations in the trapezius for (A) 5-HT and (B) norepinephrine.

active MTPs. The concentrations of these biochemicals, including protons (equivalent to inverse pH), bradykinin, SP, CGRP, TNF-α, IL-1β, 5-HT, and norepinephrine differentiate the active group from the latent and normal groups. Two additional analytes not previously sampled, IL-6 and IL-8, were also significantly higher in the active group.

We have also shown that the concentrations of these biochemicals in the upper trapezius, adjacent to active MTPs, differ quantitatively from the remote, uninvolved site we chose in the gastrocnemius muscle. There are also consistent differences in the biochemical milieu among active, latent, and normal groups in the gastrocnemius. This suggests that substances associated with pain and fatigue are not limited to local areas of MTPs or a single anatomic locus.

These findings suggest that subjects with active MTPs have a greater presence of inflammatory mediators, neuropeptides, catecholamines, and cytokines within the local milieu of the trigger point. The elevated levels of these sensitizing substances and a higher proton concentration (lower pH) in the active MTP support Simons' integrated hypothesis[1] of an area of relative local ischemia and hypoxia compared to latent and normal muscle.

According to the integrated hypothesis, "... a central MTrP [myofascial trigger point] has multiple muscle fibers with endplates releasing excessive acetylcholine, and it shows histopathological evidence of regional sarcomere shortening."[12(p100)] Sarcomere shortening requires high oxygen to maintain continuous muscle contractile activity. The combination of this increased metabolic demand and the ischemia from compromised circulation caused by increased tension of the involved sarcomeres could account for the severe local hypoxia. Consequently, the relative ischemia and hypoxia would cause the elevated levels of sensitizing substances we found in active MTPs. Furthermore, relatively higher levels of these substances in active MTPs help explain the local tenderness and referred pain of active MTPs.[12]

Issberner et al[13] showed a positive correlation between pain and local acidity. An acidic milieu alone (without muscle damage) is sufficient to cause profound changes in the properties of the "pain matrix" such that alterations in pH would be sufficient to modify the threshold sensitivity of the nociceptor. An acidic pH stimulates the production of bradykinin during local ischemia and inflammation; therefore, a local acidic milieu may explain the pain associated with an active MTP.

Mechanical hyperalgesia is a hallmark of an MTP. Ongoing nociceptive activity, however, is not necessary to cause mechanical hyperalgesia. In a rat model,[14] repeated injections of acidic saline boluses into 1 gastrocnemius muscle produced bilateral, long-lasting mechanical hypersensitivity (ie, hyperalgesia) of the paw. Furthermore, the study found that the persistent mechanical hyperalgesia was not caused by muscle tissue damage and was not maintained by continued nociceptive input from the site of muscle injury.[14] This model clearly demonstrates that secondary mechanical hyperalgesia may be maintained by neuroplastic changes in the central nervous system (eg, in spinal dorsal neurons and thalamic neurons).[14] Hong et al[15] suggest that an integrative mechanism at the spinal cord level in response to sensitized nociceptors has a role in development of active MTPs and should be considered in any pathogenetic hypotheses.

In an expansion of Simons' integrated hypothesis[1], Gerwin et al[16] postulate that the acidic pH may also modulate the motor endplate by inhibiting acetylcholinesterase. This would result in an increased concentration of acetylcholine at the synaptic cleft that would lead to sarcomere contraction and formation of a taut band.

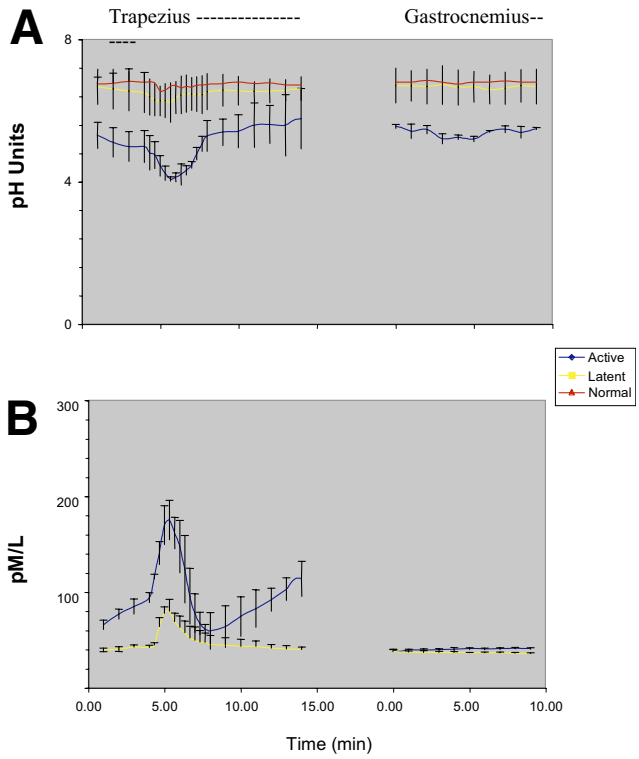

Fig 7. Analyte concentrations in the trapezius compared with the gastrocnemius for (A) pH and (B) bradykinin.

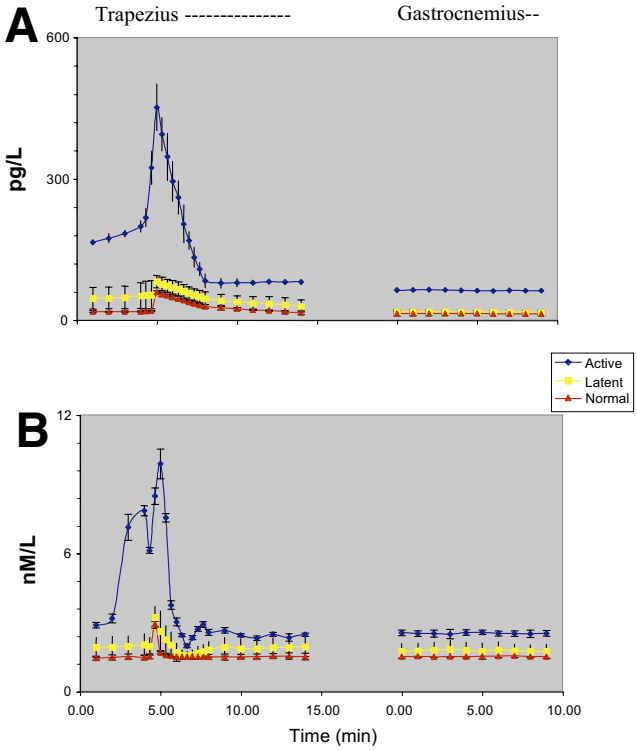

Fig 8. Analyte concentrations in the trapezius compared with the gastrocnemius for (A) SP and (B) norepinephrine.

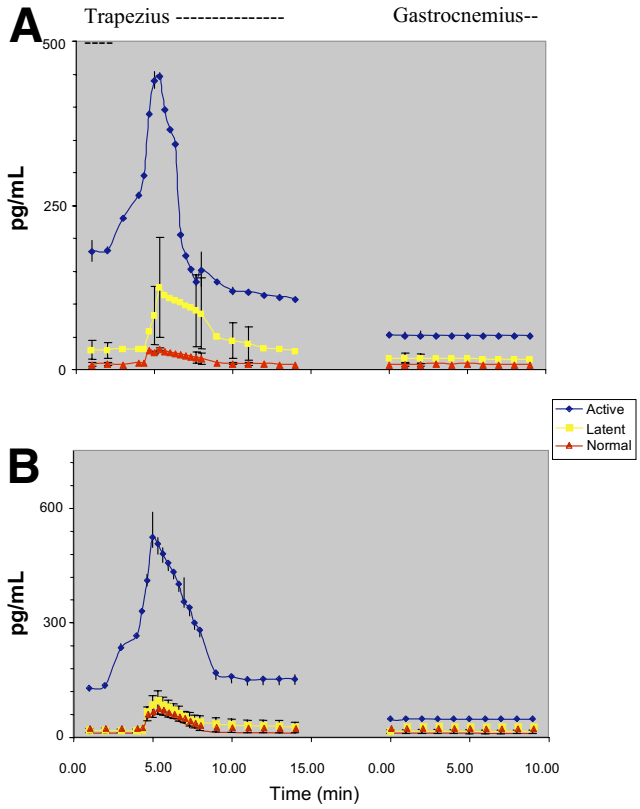

Fig 9. Analyte concentrations in the trapezius compared with the gastrocnemius for (A) TNF-α and (B) IL-6.

We found significantly elevated levels of SP and CGRP in the vicinity of the active MTPs. The orthodromic and antidromic release of these substances is greatly increased in response to nociceptor activation, (eg, by H^+ and bradykinin binding to their matched receptors).[17] This dynamic phenomenon may lead to neuroplastic changes in the dorsal horn and profound changes in neuronal activity and the perception of pain.

The goal of trigger point needling of the active MTP is to elicit multiple LTRs.[4,18] In our active group, SP and CGRP were the only 2 analytes for which concentrations during the recovery period (post) after the LTR were significantly below their concentrations at baseline (pre). This corresponds with the commonly observed decrease (at least temporarily) in pain and local tenderness after the release of an MTP by needling. Physiologically, this may be caused by interference with nociceptor membrane channels or transport mechanisms associated with a briefly augmented inflammatory response. The levels of these analytes may also fall because of a local increase in blood flow. Additional study of these analytes would be needed to explain the nature of this phenomenon.

SP causes mast cell degranulation with the release of histamine, serotonin, and upregulation of both proinflammatory (eg, TNF-α, IL-6) and anti-inflammatory cytokines (eg, IL-4, IL-10). TNF-α is the only cytokine prestored in the mast cell and is released immediately after mast cell degranulation. TNF-α stimulates norepinephrine production. We found significantly elevated levels of serotonin and norepinephrine in subjects with active MTPs. The increased levels of norepinephrine may be associated with increased sympathetic activity in the motor end plate region.

Levels of TNF-α and IL-1β were significantly elevated in the trapezius of subjects with active MTPs. In a rat model, TNF-α produces a time- and dose-dependent muscle hyperalgesia within several hours after injection into the gastrocnemius or biceps brachii muscles. This hyperalgesia was completely reversed by systemic treatment with the nonopioid analgesic metamizol.[19] Furthermore, TNF-α did not cause histopathologic tissue damage or motor dysfunction. One day after injection of TNF-α, there were elevated levels of CGRP, nerve growth factor, and prostaglandin E_2 in the muscle. Therefore, TNF-α and other proinflammatory cytokines such as IL-1β may have a role in the development of muscle hyperalgesia, and the targeting of proinflammatory cytokines might be beneficial for the treatment of muscle pain syndromes.[19] This may depend, however, on the time course of the injury and inflammatory response. Hoheisel's data[20] suggest that TNF-α has a dual action when released intramuscularly. Specifically, "... it suppresses neuronal hyperexcitability early after release but contributes to neuronal hyperexcitability in a later phase."[20(p174)]

We found significantly elevated levels of IL-6 and IL-8 in the trapezius of the active group. IL-6 has both pro- and anti-inflammatory effects and like TNF-α, IL-1β, and IL-8, it produces a dose- and time-dependent mechanical hypernoci-

Table 2: Significant Analyte Differences in Gastrocnemius, Comparison of Groups (average at 2 and 3min)

Analyte	Differences	α-Level (P)
pH	Active < latent, normal	<.01
SP, CGRP, TNF-α IL-1β, IL-6, IL-8, norepinephrine	Active > latent, normal	<.01
Bradykinin	Active > normal	<.01
5-HT	Active > latent > normal	<.05

Table 3: Analyte Concentrations at the Trapezius Time Levels (pre, peak, post) Compared With Gastrocnemius Concentrations

	Trapezius Versus Gastrocnemius		
Analytes by Group	Pre*	Peak*	Post*
Active group			
pH	NS	T<G	NS
SP, bradykinin, TNF-α, IL-1β, IL-6, IL-8, 5-HT, norepinephrine	T>G	T>G	T>G
CGRP	T>G	T>G	NS
Latent group			
pH	NS	T<G	NS
Bradykinin, IL-1β, IL-8, 5-HT, norepinephrine	NS	T>G	NS
SP, CGRP, TNF-α	T>G	T>G	T>G
IL-6	NS	T>G	T>G
Normal group			
pH	NS	NS	NS
CGRP, IL-1β, IL-6, IL-8	NS	T>G	NS
SP, 5-HT	NS	T>G	T>G
Bradykinin	T>G	T>G	NS
TNF-α, norepinephrine	NS	NS	NS

NOTE. See table 1 for definitions of time levels.
Abbreviations: G, gastrocnemius; NS, not significant; T, trapezius.
*All differences significant at $P<.05$.

ception in rats. Cytokines and chemokines play a crucial role in mediating inflammatory and neuropathic pain in rat models of tissue injury. Furthermore, the cascade of cytokines released follows 2 distinct pathways with different final mediators (ie, prostanoids and sympathetic amines). For example, "... TNF-α, IL-6, and IL-1β sequentially precede the release of prostanoids to induce hypernociception in rats."[21(p123)]

Conversely, the chemokine IL-8 participates in a separate (ie, cyclo-oxygenase [COX] independent) pathway and coordinates the sympathetic components of the inflammatory hypernociception after tissue injury. Moreover, IL-8 induces a dose- and time-dependent mechanical hypernociception. Elevated levels of IL-8 in active MTPs may mediate inflammatory hypernociception, muscle tenderness, and pain through this pathway, which is inhibited by β adrenergic receptor antagonists; COX inhibitors, however, do not inhibit this pathway.[21]

Analysis of samples taken from both muscles found that all analyte concentrations in the trapezius of the actives were generally higher than concentrations in their gastrocnemius muscles. There were fewer differences in the latent group and almost none in the normal group. The differences between the active and the other groups in the trapezius were more dramatic (ie, greater slope of the curve and higher peak and amplitude) than the differences in the gastrocnemius muscle. Concentrations in the gastrocnemius measurements remained relatively constant over the 10-minute sampling period.

The striking differences between analyte concentrations in the trapezius versus gastrocnemius muscles might be explained by several inherent muscle properties and functions. The trapezius and gastrocnemius have different muscle fiber types, mechanical properties, functions, and structures. Given the nature and density of the gastrocnemius muscle, analyte concentration locally may be insufficient to generate a noxious stimulus sufficient to produce a substantial rise in inflammatory analytes. Additionally, the trapezius is among the muscles most commonly affected by MPS because of its unique antigravity function. This puts it at high risk for overuse and susceptibility to irritation.

The trapezius and gastrocnemius muscles demonstrate different responses to needle insertion. The reaction during the pre-time level also suggests sensitivity to the needle, with the actives having the largest response, the latents a smaller response, and the normals having the least response to needle insertion. Additionally, the gastrocnemius muscles of all groups showed the least response to needle insertion. This may be associated with hyperirritability of the trapezius muscle in people with active MTPs, which is not evident in normals or in the gastrocnemius muscle.

Concentrations at the post-time level represent a recovery phase after a rapid drop in analyte levels associated with needle advancement. We thought that gastrocnemius concentrations would represent baseline levels for unaffected muscle and that post-trapezius data would trend toward these unaffected muscle levels. This was not the case in the trapezius of the actives, where all analytes except CGRP remained significantly higher than in the gastrocnemius in the recovery phase (post-level, approximately 5 minutes after needle advancement) (see table 1). This suggests that the muscle with an active MTP is more biochemically reactive to needle perturbation than latent or normal muscle tissue.

Although there were no trigger points in the upper medial gastrocnemius, our results indicate that even in this muscle, analyte levels were always significantly higher in the active group than in the normal group and generally higher than in the latent group. This suggests that analyte abnormalities may not be limited to local areas of active (painful) MTPs in the upper trapezius, but are present in unaffected muscles remote from the active MTP, although at a lower level than in the trapezius. The slightly elevated analyte levels in the gastrocnemius may be a widespread phenomenon in the active group. This may be related to a central sensitization in the active group, which lowers the threshold to stimuli and leads to a higher sensitivity to mechanical stimuli at the gastrocnemius, or to a systemic susceptibility to inflammation. There is a possibility that widespread elevation of analytes is a precursor to development of active (painful) MTPs. Conversely, people who are predisposed to develop active MTPs might have elevated baseline levels in muscles throughout their bodies. An ongoing natural history study could determine whether the relatively elevated analyte levels in the gastrocnemius in the active group follow the development of an active MTP, or if there is a baseline low-level elevation of these analytes that precedes the development of an active MTP.

We believe that further research in this area should include studying the natural history of subjects with normal muscle and those with MTPs. This type of research will determine whether MTPs resolve spontaneously or evolve into the active forms from latent or normal conditions. Microdialysis sampling of the levels of inflammatory mediators, neuropeptides, catecholamines, pro-inflammatory cytokines, and other substances (eg, anti-inflammatory cytokines, peripheral opioids) may lead to an improved biochemical characterization of the MTP and identify people who are at risk for developing persistent symptoms. Furthermore, discovering whether and which measurable substances are predictive of pain could lead to focused therapies.

Study Limitations

There are several limitations to this study. First, the sample size was small. Because of this, statistical power was low, which would result in an increased probability of a type II error. A type II error, however, can only occur when findings do not achieve significance, so we could not have made this error. Further, we

used conservative analysis (Bonferroni adjustment) to minimize type I error (ie, inappropriate significant differences), and we are therefore confident that our differences are real.

The depth of needle penetration was not standardized intersubject or within subjects because a stylus was not used. There was, however, a consistent initial end point in all groups, as determined by a change in tissue resistance at needle contact with the muscle. A consistent second end point for the active and latent groups was determined by the LTR and was approximated in the normal group as closely as possible.

We studied only 2 muscles. There is a possibility that other muscles might provide different findings. We believe, however, that the trapezius and gastrocnemius muscles are reasonable models for more generalized conclusions.

The physical findings and symptoms still may have some degree of clinical variance, as evidenced by the subject who was disqualified post hoc. There may be many different, and as yet undetermined, physiologic or pharmacologic phenomena present, and future studies will need to be specifically designed to seek these answers.

CONCLUSIONS

Our microdialysis system, utilizing samples of less than 1μL, is capable of continuous, near real-time, in vivo recovery of molecules 75kDa and smaller directly from the soft tissue environment without harmful effects on subjects.

There is a unique biochemical milieu of substances associated with pain and inflammation in the vicinity of an active MTP in the upper trapezius that includes elevated concentrations of protons, SP, CGRP, bradykinin, TNF-α, IL-1β, IL-6, IL-8, 5-HT, and norepinephrine. Concentrations of analytes from the milieu of the upper trapezius differ quantitatively from a remote uninvolved site in the medial gastrocnemius muscle. Furthermore, compared with the other groups, subjects with active MTPs in the trapezius had elevated levels of inflammatory mediators, neuropeptides, catecholamines, and cytokines in the gastrocnemius muscle. This suggests that elevations of biochemicals associated with pain and inflammation may not be limited to localized areas of active MTPs.

APPENDIX 1: COLLECTION SCHEDULE FOR DIALYSATE SAMPLES

Minute	Collections
0	Needle inserted
1	Sample (1) at minute 1
2	Sample (2) at minute 2
3	Sample (3) at minute 3
4	Samples (4−6) at minute 4 , 4:20, 4:40
5	Samples (7−9) at minute 5, 5:20, 5:40 (needle advanced into muscle at ≈5:05)
6	Samples (10−12) at 6:00, 6:20, 6:40
7	Samples (13−15) at 7:00, 7:20, 7:40
8	Sample (16) at minute 8
9	Sample (17) at minute 9
10	Sample (18) at minute 10
11	Sample (19) at minute 11
12	Sample (20) at minute 12
13	Sample (21) at minute 13
14	Sample (22) at minute 14
	Needle removed

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Suppliers

a. Pain Diagnostics & Therography, 233 E Shore Rd, Ste 108, Great Neck, NY 11023-2433.
b. Orion, 16117 Covello St, Van Nuys, CA 91406.